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<p>(54) Title: NOVEL METHOD AND PHAGE FOR THE IDENTIFICATION OF NUCLEIC ACID SEQUENCES ENCODING MEMBERS OF A MULTIMERIC (POLY)PEPTIDE COMPLEX</p> <div style="display: flex; justify-content: space-around;"> <div data-bbox="370 1150 760 1759"> <p>General description of the polyphage principle</p> </div> <div data-bbox="841 1150 1230 1759"> <p>General description of the polyphage principle (cont.)</p> </div> </div> <p>(57) Abstract</p> <p>The present invention relates to methods for the identification of nucleic acid sequences encoding members of a multimeric (poly)peptide complex by screening for polyphage particles. Furthermore, the invention relates to products and uses thereof for the identification of nucleic acid sequences in accordance with the present invention.</p>		

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NOVEL METHOD AND PHAGE FOR THE IDENTIFICATION OF NUCLEIC ACID SEQUENCES ENCODING MEMBERS OF A MULTIMERIC (POLY)PEPTIDE COMPLEX

The present invention relates to methods for the identification of nucleic acid sequences encoding members of a multimeric (poly)peptide complex by screening for polyphage particles. Furthermore, the invention relates to products and uses thereof for the identification of nucleic acid sequences in accordance with the present invention.

Since its first conception by Ladner in 1988 (WO88/06630), the principle of displaying repertoires of proteins on the surface of phage has experienced a dramatic progress and has resulted in substantial achievements. Initially proposed as display of single-chain Fv (scFv) fragments, the method has been expanded to the display of bovine pancreatic trypsin inhibitor (BPTI) (WO90/02809), human growth hormone (WO92/09690), and of various other proteins including the display of multimeric proteins such as Fab fragments (WO91/17271; WO92/01047).

A Fab fragment consists of a light chain comprising a variable and a constant domain (VL-CL) non-covalently binding to a heavy chain comprising a variable and constant domain (VH-CH1). In Fab display one of the chains is fused to a phage coat protein, and thereby displayed on the phage surface, and the second is expressed in free form, and on contact of both chains, the Fab assembles on the phage surface.

Various formats have been developed to construct and screen Fab phage-display libraries. In its simplest form, just one repertoire, e. g. of heavy chains, is encoded on the phage or phagemid vector. A corresponding light chain, or a repertoire of light chains, is expressed separately. The Fab fragments assemble either inside a host cell, if the light chain is co-expressed from a plasmid, or outside the cell in the medium, if a collection of secreted phage particles each displaying a heavy chain is contacted with the light chain(s) expressed from a different host cell. By screening such Fab libraries, just the information about the heavy chain encoded on the phage or phagemid vector is retrievable, since that vector is packaged in the phage particle. By reverting the format and displaying a library of light chains, and

assembling Fab fragments by co-expressing or adding on or more of the heavy chains identified in the first round, corresponding light chain-heavy chain pairs can be identified.

To avoid that multi-step procedure, both repertoires may be cloned into one phage or phagemid vector, one chain expressible as a fusion with at least part of a phage coat protein, the second expressible in free form. After selection, the phage particle will contain the sequence information about both chains of the selected Fab fragments. The disadvantage of such a format is that the overall complexity of the library is limited by transformation efficiency. Therefore, the library size will usually not exceed 10^{10} members.

For various applications, a library size of up to 10^{14} would be advantageous. Therefore, methods of using site-specific recombination, either based on the Cre/lox system (WO92/20791) or on the att λ system (WO 95/21914) have been proposed. Therein, two collection of vectors are sequentially introduced into host cells. By providing the appropriate recombination sites on the individual vectors, recombination between the vectors can be achieved by action of an appropriate recombinase or integrase, achieving a combinatorial library, the overall library size being the product of the sizes of the two individual collections. The disadvantages of the Cre/lox system are that the recombination event is not very efficient, it leads to different products and is reversible. The att λ system leads to a defined product, however, it creates one very large plasmid which has a negative impact on the production of phages. Furthermore, the action of recombinase or integrase most likely leads to undesired recombination events.

Thus, the technical problem underlying the present invention is to develop a simple, reliable system which enables the simultaneous identification of members of a multimeric (poly)peptide complex, such as the identification of heavy and light chain of a Fab fragment, in phage display systems.

The solution to this technical problem is achieved by providing the embodiments characterized in the claims. Accordingly, the present invention allows to easily create and screen large libraries of multimeric (poly)peptide complexes for properties such as binding to a target, as in the case of screening Fab fragment libraries, or such as enzymatic activity, as in the case of libraries of multimeric enzymes. The technical approach of the present invention, i.e. the retrieval of information about two members of a multimeric (poly)peptide complex

encoded on two different vectors without requiring a recombination event, is neither provided nor suggested by the prior art.

Accordingly, the present invention relates to a method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes, said method being characterized by screening or selecting for polyphage particles that contain said combination.

Surprisingly, it has been achieved by the present invention that the phenomenon of polyphages can be used to co-package the genetic information of two or more members of multimeric (poly)peptide complexes in a phage display system. The occurrence of polyphage particles has been observed 30 years ago (Salivar et al., Virology 32 (1967) 41-51), where it was described that approximately 5% of a phage population form particles which are longer than unit length and which contain two or more copies of phage genomic DNA. They occur naturally when a newly forming phage coat encapsulates two or more single-stranded DNA molecules. In specific cases, it has been seen that co-packaging of phage and phagemids or single-stranded plasmid vectors takes place as well (Russel and Model, J. Virol. 63 (1989) 3284-3295). Despite of occasional scientific articles about the morphogenesis of polyphage particles, a practical application has never been discussed or even been mentioned. In WO92/20791 in example 26, a model experiment for a combinatorial Fab display library expressed from separate vectors is presented. However, there is only a screening process for either of the two vectors described. Thus, the prior art teaches away from screening for the simultaneous presence of two vectors in a polyphage particle.

In the context of the present invention, the term "multimeric (poly)peptide complex" refers to a situation where two or more (poly)peptide(s) or protein(s), the "members" of said multimeric complex, can interact to form a complex. The interaction between the individual members will usually be non-covalent, but may be covalent, when post-translational modification such as the formation of disulphide-bonds between any two members occurs. Examples for "multimeric (poly)peptide complexes" comprise structures such as fragments derived from immunoglobulins (e. g. Fv, disulphide-linked Fv (dsFv), Fab fragments), fragments derived from other members of the immunoglobulin superfamily (e.g. α , β -

heterodimer of the T-cell receptor), and fragments derived from homo-or heterodimeric receptors or enzymes. In phage display, one of said members is fused to at least part of a phage coat protein, whereby that member is displayed on, and assembly of the multimeric complex takes place at, the phage surface. A "combinatorial phage library" is produced by randomizing at least two members of said multimeric (poly)peptide complex at least partially on the genetic level to create two libraries of genetically diverse nucleic acid sequences in appropriate vectors, by combining the libraries in appropriate host cells and by achieving co-expression of said at least two libraries in a way that a library of phage particles is produced wherein each particle displays one of the possible combinations out of the two libraries. By screening such a combinatorial phage library displaying multimeric (poly)peptide complexes for a predetermined property, a collection of phage particles will be identified. Partially, these particles will just contain the genetic information of one of the members of the multimeric complex. The inventive principle of the present invention is the screening step for polyphage particles containing the genetic information of a combination of library members.

Furthermore, the present invention relates to a method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes, comprising the steps of

- (a) providing a first library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to, and displayed at, the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;
- (b) providing a second library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules of said second library are able to be packaged in a phage particle and carry

or encode a second selectable and/or screenable property different from said first property;

- (c) optionally, providing nucleic acid sequences encoding further members of a multimeric (poly)peptide complex;
- (d) expressing members of said libraries of recombinant vectors mentioned in steps (a), (b), and optionally nucleic acid sequences mentioned in step (c), in appropriate host cells under appropriate conditions, so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;
- (e) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;
- (f) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;
- (g) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (f);
- (h) identifying said combination of nucleic acid sequences.

Optionally, further members of said multimeric complex may be provided in the case of ternary, quaternary or higher (poly)peptide complexes. These further members may, for example, be co-expressed from one of the phage or phagemid vectors or from a separate vector such as a plasmid. Even libraries of such further members could be employed in which case further screenable or selectable properties would have to be introduced on the corresponding vectors. Alternatively, such further libraries could be contained in said first or second libraries of recombinant vector molecules. In another option, further screening and/or selection steps or a repetition of the individual steps can be carried out, to optimize the result of obtaining and identifying said nucleic acid sequences.

Furthermore, the present invention relates to a method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes, comprising the steps of

- (a) expressing in appropriate host cells under appropriate conditions

- (aa) genetically diverse nucleic acid sequences contained in a first library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to and displayed at the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;
 - (ab) genetically diverse nucleic acid sequences contained in a second library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules are able to be packaged in a phage particle and carry or encode a second selectable and/or screenable property different from said first property;
 - (ac) optionally, nucleic acid sequences encoding further members of a multimeric (poly)peptide complex,
- so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;
- (b) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;
 - (c) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;
 - (d) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (c);
 - (e) identifying said combination of nucleic acid sequences.

In a preferred embodiment of the method of the present invention, the vectors of said first and said second library are a combination of a phage vector and a phagemid vector.

In a further preferred embodiment of the method of the present invention, the vectors of said first and said second library are a combination of two phagemid vectors, said appropriate conditions comprising complementation of phage genes by a helper phage.

In a most preferred embodiment of the method of the present invention said two phagemid vectors are compatible.

The term "compatibility" refers to a property of two phagemids to be able to coexist in a host cell. Incompatibility is connected to the presence of incompatible plasmid origins of replication belonging to the same incompatibility group. An example for compatible plasmid origins of replication is the high-copy number origin ColE1 and the low-copy number origin p15A.

Therefore, in a further preferred embodiment of the method of the present invention, said two phagemid vectors comprise a ColE1 and a p15A plasmid origin of replication.

In a most preferred embodiment of the method of the present invention, said two phagemid vectors comprise a ColE1 and a mutated ColE1 origin.

It could be shown, that two phagemids both having a ColE1-derived plasmid origin of replication can coexist in a cell as long as one of the ColE1 origins carries a mutation.

Particularly preferred is a method, wherein said vectors and/or said helper phage comprise different phage origins of replication.

Most preferred is an embodiment of the method of the present invention, wherein said phage vector, said phagemid vector(s) and/or said helper phage are interference resistant.

The term "interference" refers to a property that phagemids inhibit the production of progeny phage particles by interfering with the replication of the DNA of the phage. "Interference resistance" is a property which overcomes this problem. It has been found that mutations in the intergenic region and/or in gene II contribute to interference resistance (Enea and Zinder, Virology 122 (1982), 222-226; Russel et al., Gene 45 (1986) 333-338). It was identified that phages called IR1 and IR2 (Enea and Zinder, Virology 122 (1982), 222-226), and mutants derived therefrom such as R176 (Russel and Model, J. Bacteriol. 154 (1983) 1064-1076), R382, R407 and R408 (Russel et al., Gene 45 (1986) 333-338) and R383 (Russel and Model, J. Virol. 63 (1989) 3284-3295) are interference resistant by carrying mutations in the untranslated region upstream of gene II and in the gene II coding region.

Therefore, in a preferred embodiment of the method of the present invention, said phage vector, said phagemid vector(s) and/or said helper phage have mutations in the phage intergenic region(s), preferably in positions corresponding to position 5986 of f1, and/or in gene II, preferably in positions corresponding to position 143 of f1.

In a most preferred embodiment said phage vector, said phagemid vector(s) and/or said helper phage are, or are derived from, IR1 mutants such as R176, R382, R383, R407, R408, or from IR2 mutants.

In a further embodiment of the method of the invention, said vectors and/or said helper phage comprise hybrid nucleic acid sequences of f1, fd, and/or M13 derived sequences.

In the context of the present invention, the term "hybrid nucleic sequences" refers to vector elements which comprise sequences originating from different phage(mid) vectors.

Surprisingly, it has been found that a vector constructed combining a part derived from fd phage and a second part derived from R408, a derivative of f1 phages, is interference resistant and additionally, gives predominantly polyphage particles.

Therefore, a most preferred embodiment of the method of the present invention relates to a vector which is, or is derived from, fpep3_1B-IR3seq with the sequence listed in Figure 4.

In a yet further preferred embodiment of the method according to the present invention, said derivative is a phage comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

The invention relates in an additional preferred embodiment to a method, wherein said derivative is a phagemid comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

The invention relates in a further preferred embodiment to a method, wherein said derivative is a helper phage comprising essentially the phage origin or replication from fpep3_1B-

IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Most preferred is an embodiment of the method of the invention, wherein said derivatives comprise the combined fd/fl origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.

The formation of polyphage particles has been examined in more detail by different groups. It was found that amber mutations in genes VII and IX lead to the amplified production of infectious polyphage particles (Lopez and Webster, Virology 127 (1983) 177-193). A couple of mutants in gene VII (R68, R100) and in gene IX (N18) were identified and further characterized.

Accordingly, in a preferred embodiment of the method of the present invention, the gene VII contained in any of said vectors contains an amber mutation, and most preferably, said mutation is identical to those found in phage vectors R68 or R100.

Further preferred is an embodiment, wherein the gene IX contained in any of said vectors contains an amber mutation, and most preferably said mutation is identical to that found in phage vector N18.

Several phage coat proteins have been used in displaying foreign proteins including the gene III protein (gIIIp), gVIp, and gVIIIp.

In a preferred embodiment of the method of the present invention, said phage coat protein is gIIIp or gVIIIp.

In a particularly preferred embodiment of the method of the present invention, said phage particles are infectious by having a full-length copy of gIIIp.

The gIIIp is a protein comprising three domains. The C-terminal domain is responsible for membrane insertion, the two N-terminal domains are responsible for binding to the F pilus of *E. coli* (N2) and for the infection process (N1).

In a most preferred embodiment of the method of the invention, said phage particles are non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the

displayed multimeric (poly)peptide complexes with a corresponding partner coupled to an infectivity-mediating particle.

In the context of the present invention, the term "infectivity-mediating particle" (IMP) refers to a construct comprising either the N1 domain or the N1-N2 domain. On interaction with a non-infectious phage lacking said domains, infectivity of the phage particles can be restored. The interaction between the non-infectious phage and the IMP can be mediated by a ligand fused to the IMP, which can bind to a partner displayed on the phage. By screening a non-infectious phage display library against a target ligand-IMP construct, restoration of infectivity can be used to select target-binding library members.

In a further preferred embodiment of the method of the invention, said truncated gIIIp comprises the C-terminal domain of gIIIp.

In a yet preferred embodiment of the method of the invention, said truncated gIIIp is derived from phage fCA55.

In addition to the work by Lopey and Webster cited above, Crissman and Smith (Virology 132 (1984) 445-455) could show, that the phage fCA55 which has a large deletion in gene III removing the N-terminal domains and a large part of the C-terminal domain leads exclusively to the formation of polyphages.

Particularly preferred is an embodiment of the method of the invention, wherein said predetermined property is binding to a target.

In a preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is a fragment of an immunoglobulin superfamily member.

In a most preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is a fragment of an immunoglobulin.

In a further most preferred embodiment of the method of the invention, said fragment is an Fv, dsFv or Fab fragment.

An additional preferred embodiment of the present invention relates to a method, wherein said predetermined property is the activity to perform or to catalyze a reaction.

In a preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is an enzyme.

In a most preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is a fragment of a catalytic antibody.

In a further most preferred embodiment of the method of the invention, said fragment is an Fv, dsFv or Fab fragment.

An additional preferred embodiment of the invention relates to a method, wherein selectable and/or screenable property is the transactivation of transcription of a reporter gene such as beta-galactosidase, alkaline phosphatase or nutritional markers such as his3 and leu, or resistance genes giving resistance to an antibiotic such as ampicillin, chloramphenicol, kanamycin, zeocin, neomycin, tetracycline or streptomycin.

In a most preferred embodiment of the method of the invention, said generation of said first and second screenable and/or selectable property is achieved after infection of appropriate host cells by said collection of phage particles.

Particularly preferred is a method, wherein said identification of said nucleic acid sequences is effected by sequencing.

Further preferred is a method, wherein said host cells are E.coli XL-1 Blue, K91 or derivatives, TG1, XL1kann or TOP10F.

An additional preferred embodiment of the invention relates to a polyphage particle which

(a) contains

(i) a first recombinant vector molecule that comprises a nucleic acid sequence, which encodes a fusion protein of a first member of a multimeric (poly)peptide complex

fused to at least part of a phage coat protein, and that carries or encodes a first selectable and/or screenable property, and

(ii) a second recombinant vector molecule that comprises a nucleic acid sequence, which encodes a second member of a multimeric (poly)peptide complex, and that carries or encodes a second selectable and/or screenable property different from said first property;

and (b) displays said multimeric (poly)peptide complex at its surface.

A most preferred embodiment of the invention relates to a polyphage particle, wherein said phage coat protein is the gIIIp.

A further preferred embodiment of the present invention relates to a polyphage particle which is infectious by having a full-length copy of gIIIp present, either in said fusion protein, or in an additional wild-type copy.

Additionally, the invention relates to a polyphage particle which is non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the displayed multimeric (poly)peptide complex with a corresponding partner coupled to an infectivity-mediating particle.

Most preferably, the invention relates to the phage vector fpep3_1B-IR3seq with the sequence listed in Figure 4.

Additionally preferred, the invention relates to a phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Further preferred is an embodiment of the invention, which relates to a phagemid vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Preferably, the invention relates to a helper phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Additionally preferred is an embodiment, said derivatives comprise the combined fd/fl origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.

Further preferred is the use of any of the vectors according to the present invention in the generation of polyphage particles containing a combination of at least two different vectors.

Most preferred is the use of vectors of the invention, wherein said combination of different vectors comprises nucleic acid sequences encoding members of a multimeric (poly)peptide complex.

Further preferred in the present invention is the use of vectors, wherein said combination of different vectors comprises nucleic acid sequences encoding interacting (poly)peptides/proteins.

Legends to Figures:

Figure 1: General description of the polyphage principle for the display of a Fab library: e.g. library 1: library of VL chains; library 2: VH chains; both libraries on compatible phagemids; in a: libraries are transformed into host cells; in b: library 1 is rescued by a helper phage; in c: libraries are combined by infection; in d: co-expression of heavy and light chains; in e: rescue by helper phages, production of phage particles, assembly of Fab on phage, selection for target; note 1: A certain fraction of the phage particles will be normal unit-length particles containing just one of the two genomes (not shown in Figure 1). Furthermore, polyphage does not discriminate which genomes to package. Therefore, the combinations shown in Figure 1 can arise. To select for

correctly packaged genomes, the subsequent steps are required; in f: infect host cells; in g: select for ability to confer resistance to two antibiotics to infected cells; note 2: only phage that satisfy condition according to g) represent polyphage particles which contain the correct combination of heavy and light chain of binding Fabs (Hetero-polyphage). Unit-length phage as well as polyphage carrying two identical genomes will confer only resistance to one antibiotics.

- Figure 2: Functional map and sequence of phage vector fhag1A
- Figure 3: Functional map and sequence of phage vector fjun_1B
- Figure 4: Functional map and sequence of phage vector fpep3_1B-IR3seq
- Figure 5: Compatibility of various phage and phagemid vectors: co-transformation of different vector pairs and growth in liquid culture (can/amp selection):
 A. fjun_1B-R408-IR/pIG10_pep10; B. fjun_1B/pIG10_pep10 (only 1 colonie);
 C. fpep3_1B-IR3/pIG10_pep10; D. fjun_1B-R408-IR/pOK1Djun; E. fjun_1B/pOK1Djun: no growth; F. fpep3_1B-IR3/pOK1Djun;
 a. fjun_1B; b. fjun_1B-R408-IR; c. fpep3_1B-IR3; d. pIG10_pep10; e. pOK1Djun
- Figure 6: co-transformation of positive (pep3/p75ICD combination, lane 9) and negative (jun/p75ICD, lane 10) pairs; lane 1 to 8: SIP transductants
- Figure 7: Sensitivity of SIP hetero-polyphage system for selection in solution: #SIP hetero-polyphage transductants, transducing units (t.u.)/ml, produced by co-cultures of co-transformants as in Figure 6 mixed at the indicated ratios.
- Figure 8: PCR to identify phage vector(s) present in SIP polyphage transductants: lane 1 to 6: SIP polyphage transductants; lane A: fpep3_1B-IR3/pIG10.3-IMPp75 co-transformant; lane B: fjun_1B-IR3/pIG10.3-IMPp75 co-transformant
- Figure 9: IR Phage and Phagemid are Co-packaged into Polyphages: 1: Δ gIII phage + gIII plasmid; 2: IR phage+ phagemid
- Figure 10: SIP Information is Co-transduced by Polyphages: a: IMPp75 on phage vector; b: pep10-gIII-CT fusion on phage vector; c: IMPp75 on phagemid vector; d: pep10-gIII-CT fusion on phagemid vector

The examples illustrate the invention

Example 1: Selection for polyphage transductants

In WO92/01047, page 83, a model experiment for a two-vector system is described which uses a phage vector (fd-CAT2-IV) encoding a light chain and a phagemid vector (pHEN1-III) encoding a heavy chain. The phagemid, grown in *E. coli* HB2151, was rescued with fd-CAT2-IV phage, and functional phage(mid)s produced. By infecting TG1 cells and plating on tetracycline (to select for fd-CAT) and ampicillin (to select for pHEN1), the ratio of phage and phagemid being packaged was determined.

By repeating this experiment, but plating on TYE plates with both antibiotics, polyphage transductants transducing both resistances simultaneously can be selected, and the genetic information contained on the phage and phagemid vector can be retrieved.

By replacing the single light and heavy chain in the constructs mentioned above by corresponding repertoires, a library of Fab-displaying phage particles can be produced. By screening that library against an immobilized target, a collection of phage particles can be identified. Polyphage particles contained in that collection can be identified by transducing both resistances as described above.

Example 2: Generation and use of an interference-resistant filamentous phage to co-package the genetic information of co-displayed interacting proteins

Introduction

The physical connection of randomly combined genetic information is of vital importance in processes such as interactive screening of two libraries of expressed protein members or for co-expression and co-display of protein pairs which are dependent on the interaction with each other for proper function.

2.1.: Construction of a interference resistant filamentous phage:

2.1.1.: Construction of fjun_1B:

- fhag1A (see Figure 2)

- a. The phage vector f17/9-hag (Krebber *et al.*, 1995, *FEBS Letters* 377, 227-231) is digested with EcoRV and XmnI. The 1.1 kb fragment containing the anti-HAG Ab gene is isolated

by agarose gel electrophoresis and purified with a Qiagen gel extraction kit. This fragment is ligated into a pre-digested pIG10.3 vector (EcoRV-XmnI). Ligated DNA is transformed into DH5a cells and positive clones are verified by restriction analysis. The recombinant clone is called pIGhag1A. All cloning described above and subsequently are according to standard protocols (Sambrook *et al.*, 1989, *Molecular Cloning: a Laboratory Manual*, 2nd ed.)

- b. The vector f17/9-hag (Krebber *et al.*, 1995) is digested with EcoRV and StuI. The 7.9 kb fragment is isolated and self-ligated to form the vector fhag2.
- c. The chloramphenicol resistance gene (CAT) assembled *via* assembly PCR (Ge and Rudolph, *BioTechniques* 22 (1997) 28-29) using the template pACYC (Cardoso and Schwarz, *J. Appl. Bacteriol.* 72 (1992) 289-293) is amplified by the polymerase chain reaction (PCR) with the primers:
CAT_BspEI(for): 5' GAATGCTCATCCGGAGTTC
CAT_Bsu36I(rev): 5' TTTCAGTGGCCTCAGGCTAGCACCAGGCGTTTAAG
- d. The PCR is done following standard protocols (Sambrook *et al.*, 1989). The amplified product is digested with BspEI and Bsu36I then ligated into pre-digested fhag2 vector (BspEI-Bsu36I; 7.2 kb fragment) to form fhag2C.
- e. The vector fhag2C is digested with EcoRI and the ends made blunt by filling-in with Klenow fragment. The flushed vector is self-ligated to form vector fhag2CdelEcoRI.
- f. pIGhag1A is digested with XbaI and HindIII. The 1.3 kb fragment containing the anti-HAG gene fused with the C-terminal domain of filamentous phage pIII protein is isolated and ligated with a pre-digested fhag2CdelEcoRI phage vector (XbaI-HindIII; 6.4 kb) to create the vector fhag1A.

- fjun_1B (see Figure 3)

- a. The DNA encoding the C-terminal domain including the long linker separating it from the amino terminal domain of the filamentous phage pIII (gIII short) is amplified by PCR using pOK1 (Gramatikoff *et al.*, *Nucleic Acids Res.* 22 (1994) 5761-5762) as template with the primers:
gIII short(for): 5'GCTTCCGGAGAATTCAATGCTGGCGGCGGCTCT3'
gIII short(rev): 5'CCCCCCCCAAGCTTATCAAGACTCCTTATTACG3'
- b. The PCR is done following standard protocols (Sambrook *et al.*, 1989). The amplified product is digested with EcoRI and HindIII, then ligated into pre-digested fhag1A vector (EcoRI-HindIII) to form the vector fjun_1B.

2.1.2.: Construction of fjun_1B-R408IR:

In order to introduce mutations which have been described to confer an interference resistance phenotype (Enea and Zinder, Virology 122 (1982), 222-226) into the non-interference resistant fd phage vector fjun_1B (see Fig.3), a 1.7 kb fragment of helper phage R408 (Stratagene) comprising the region between the unique restriction sites *Dra*III and *Bsr*GI was PCR amplified by assembly PCR. Subfragments of the 1.7 kb *Dra*III/*Bsr*GI fragment were amplified from the f1 phage R408 template DNA with primer combinations FR604/FR605 and FR606/FR607 to introduce via the partially complementary primers FR605 and FR606 an additional *gII* mutation found to be present in the recipient construct fjun_1B. Resulting PCR fragments were gel-purified and combined to serve as template in an subsequent assembly PCR with primers FR604 and FR607. PCR conditions were standard, with approx. 25 ng template, 10 pmole of each primer, 250 pmole of each dNTP, 2 mM Mg, 2.5 U Pfu DNA polymerase (Stratagene). Amplification was done for 30 cycles, with 1 min denaturation at 94 C, 1 min annealing at 50°C, 1 min extension at 72°C. The correct-sized 1.7 kb assembly PCR product was gel-purified, digested with *Dra*III and *Bsr*GI and cloned into *Dra*III/*Bsr*GI-digested fjun_1B, generating fjun_1B-R408IR.

Primers: FR604 5' GTTCACGTAGTGGGCCATCG 3'
 FR605 5' TGAGAGGTCTAAAAAGGCTATCAGG 3'
 FR606 5' TAGCCTTTTTAGACCTCTCAAAAATAG 3'
 FR607 5' CGGTGTACAGACCAGGCGC 3'

2.2.: Proof of principle experiments

Despite of the absence of the two originally associated IR mutations, the hybrid phage vector fjun_1B-R408IR (carrying the chloramphenicol acetyltransferase conferring chloramphenicol resistance) could be co-transformed with a phagemid (pOK1deltajun, carrying the beta-lactamase gene conferring ampicilin resistance) containing a phage origin of replication. More importantly, fjun_1B-R408IR could stably co-exist with the phagemid pOK1deltajun, and the phagemid was efficiently co-packaged together with the fjun_1B-R408IR phage genome into polyphage particles. Titers of polyphages, simultaneously

transducing chloramphenicol and ampicillin resistance, reached 6×10^8 transducing units (t.u.)/ml of overnight bacterial culture K91 plating cells, a number almost equivalent to a titer of 10^9 /ml seen after selection on chloramphenicol only. Selection of the K91 transductants on ampicillin only gave a titer of 5×10^9 /ml. These titers indicated that more than 50 % of all phages containing fjun_1B-R408IR also contained the phagemid pOK1deltajun, thus representing polyphages. This high ratio of polyphages was confirmed by restriction analysis of transductants which had been selected on chloramphenicol only. More than 50 % of these clones also contained the phagemid in addition to the fjun_1B-R408IR phage genome. fjun_1B-R408IR was isolated in pure form from an individual transductant, which contained only this phage. The construct fjun_1B-R408IR was used with pOK1deltajun for co-transformation of DH5 α cells, in order to produce selectively-infective phages (SIP) via fos-jun leucine zipper interaction (which non-covalently restores wt gIII function). Stable, double-resistant co-transformants were obtained with this combination and individual clones were grown overnight in the presence of cam/amp. The culture supernatant of these clones was filtered through a 45 μ M membrane filter and used to infect exponentially-growing F+ bacteria (K91 strain) for 20 min at 37 C. To test for the presence of infective SIP polyphages the cells were plated on LB agar plates containing cam and amp and plates were incubated at 37 C overnight. Approx. 500 to 1000 transforming units (t.u.)/ml resulting in double-resistant transductants were obtained from individual co-transformants. DNA of those transductants was analyzed by restriction analysis which showed that 95 % (15/16 clones) of the clones had the correct pattern expected for fjun_1B-R408IR and pOK1deltajun. Supernatants of several polyphage transductants were tested for persistent SIP phage production by re-infection of K91 cells. This confirmed that polyphage transductants continued to produce infective SIP phages and restriction analysis of the resulting 2nd round polyphage transductants showed that 44 % (14/32 clones) contained the correct vector combination. The rest of the clones contained the correct pOK1deltajun phagemid plus a recombined phage vector with a restored wt gIII, indicating an increase in recombination frequency when both vectors are propagated in the rec+ strain K91 (compared to the rec- strain DH5 α used for co-transformation of IR phage and phagemid). To test other protein-protein interactions which give a higher titer of infective SIP phages and to verify the presence of hetero-polyphages (co-packaging of phage and phagemid instead of co-infection by monophages or homo-polyphages), two peptide ligands (previously selected by SIP, WO97/32017)

which bind to the p75 rat neurotrophin receptor (Chao et al., Science 232 (1986) 518-521) intracellular domain (p75ICD) were cloned as N-terminal gIIIc fusions in fjun_1B-R408IR (replacing jun) and the phagemid pIG10.3, leading to constructs fpep3_1B-IR3seq and pIG10.3-pep10 (WO97/32017), respectively, which contain the peptide pep3: 5'-TGTATTGTTTATCATGCTCATTATCTTGTTGCTAAGTGT-3' encoding the amino acid sequence (CysIleValTyrHisAlaHisTyrLeuValAlaLysCys) instead of the jun sequence. Sequencing of the respective parts of the transferred R408 fragment in fpep3_1B-IR3seq revealed that neither of the two IR mutations (the G5986>A mutation from complementation group I in the gII 5' non-translated region, which should be found at position 3225 in fpep3_1B-IR3seq, and the C143>T mutation (3789 in fpep3_1B-IR3seq) from complementation group II leading to a Thr>Ile amino acid exchange in gII) were found to be present. However, the gII mutation G6090>T (3329 in fpep3_1B-IR3seq), leading to a Leu>Val exchange, introduced by assembly PCR was present. Furthermore, three additional mutations compared to an f1 phage could be identified: G5737>A (2976 in fpep3_1B-IR3seq) in the phage origin of replication, G343>A (3989) in gII, and G601>T (4247) in gII/X.

The functional map and the sequence of fpep3_1B-IR3seq are given in Figure 4. This sequence was double-checked several times. It could be shown that differences in the sequence of fpep3_1B-IR3seq compared to published sequence data could be explained by mutations already present in the starting constructs used for cloning fjun_1B-R408IR and fpep3_1B-IR3seq.

Co-transformation experiments (Fig. 5) using combinations of pIG10.3 or pOK1 phagemids (both with f1 oris) with fjun_1B ("wt" fd phage), fjun_1B-R408-IR (containing the DraIII/BsrGI fragment from R408) or fpep3_1B-IR3 (containing the DraIII/BsrGI fragment from R408 and the PCR mutation) revealed that the PCR mutation is not necessary for the IR phenotype, at least judged by the ability to be co-transformable with a phagemid and the ability of individual co-transformants to grow in liquid culture (cam/amp selection).

Additionally, the interacting protein partner p75ICD was cloned as a C-terminal fusion to the infectivity-mediating domains (N1-N2) of gIII (infectivity-mediating particle (IMP) fusion) resulting in constructs fIMPp75-IR3 and pIG10.3-IMPp75.

The IR phage was tested with the SIP pairing fpep3_1B-IR3seq3/ pIG10.3-IMPp75 (which gives a higher titer than fos/jun SIP) in the presence of the negative control combination fjun_1B-IR3seq3/ pIG10.3-IMPp75 (Fig. 6). A SIP hetero-polyphage titer of 1.5×10^5 /ml (cam/amp-resistant transductants) was achieved with fpep3_1B-IR3seq3/ pIG10.3-IMPp75. To test SIP sensitivity in a model library vs. library setting, co-transformants of fpep3_1B-IR3seq3/ pIG10.3-IMPp75 were diluted in an excess fjun_1B-IR3/ pIG10.3-IMPp75 and the supernatant of the bacterial co-culture was assayed for SIP hetero-polyphages. This showed that down to a dilution of 10^{-5} to 10^{-6} can be recovered (Fig. 7).

To prove that only the correct phage vector is present in SIP polyphage transductants, DNA of positive (fpep3_1B-IR3seq3/ pIG10.3-IMPp75) and negative (fjun_1B-IR3/ pIG10.3-IMPp75) control co-transformants, as well as DNA from the SIP polyphage transductants derived from SIP phages produced by the mix of positive and negative control bacteria was analyzed by PCR (Fig. 8). Primers FR614 (5'-GCTCTAGATAACGAGGGC-3') and FR627 (5'-CGCAAGCTTAAGACTCCT-TATTACGC-3') amplify the phage region from the start of ompA to the end of gIII. PCR products derived from fpep3_1B-IR3seq3 and fjun_1B-IR3 can be discriminated by size. Gel analysis of the above samples verified that only the expected fpep3_1B-IR3seq3 phage was present in SIP polyphage transductants (6 analyzed).

To physically demonstrate the existence of hetero-polyphages (which have phage and phagemid co-packaged) when using the IR phage vector, phages produced by co-transformants of fIR3/pIG10.3-IMPp75 and as a control fjun_1B/JB61 ("wt" phage plus complementing gIII plasmid) were separated on an agarose gel (Fig. 9). This showed that the fIR3/pIG10.3-IMPp75 combination produced substantially more slower migrating (thus bigger) phages than the fjun_1B/JB61 control combination. The ratio was almost inversed. Elution of phages from various regions of the gel and subsequent titering of the eluate on plating cells showed that the upper gel region contained a significant portion of double resistance-transducing phages which thus can be regarded as hetero-polyphages.

The pairs fpep3_1B-IR3 and pIG10.3-IMPp75 as well as fIMPp75-IR3 and pIG10.3-pep10 were co-transformed into DH5 α , individual cam/amp resistant clones were grown and the culture supernatant was tested on K91 cells for SIP phage production (Fig. 10). The combinations fpep3_1B-IR3/pIG10.3-IMPp75 and fIMPp75-IR3/pIG10.3-pep10 gave a titer of 1.5×10^5 t.u./ml and 5×10^3 t.u./ml, respectively when assayed for cam/amp-resistant transductants. The titer for each combination when assayed on LB cam was nearly the same as when assayed on LB cam/amp. This demonstrated efficient co-packaging of phage and phagemid DNA to almost 100 %, as seen before with the initial fjun_1B-R408IR and pOK1deltajun combination. To proof the existence of polyphages which individually co-transduce phage and phagemid DNA simultaneously, and to rule out the possibility of transduction of the two resistance markers by independent (and thus random) co-infection by two different phages which have only phage or phagemid packaged, a statistical test was performed. Defined, identical aliquots of bacterial culture supernatants of an individual co-transformant representing each of the two SIP vector combinations described above (fpep3_1B-IR3/pIG10.3-IMPp75 and fIMPp75-IR3/pIG10.3-pep10) were either used individually to infect K91 cells followed by selection on LB cam and LB amp plates, or the same supernatant aliquots from the two vector combinations were mixed before infection of K91 cells and selection on LB cam/amp. 117 cam-resistant, 328 amp-resistant and 141 cam/amp-resistant transforming units were present in the supernatant aliquot from the fIMPp75-IR3/pIG10.3-pep10 combination and 40 cam-resistant, 30 amp-resistant and 23 cam/amp-resistant transforming units were present in the supernatant aliquot from the fpep3_1B-IR3/pIG10.3-IMPp75 combination. The mix of both supernatant aliquots contained 166 cam-resistant and 162 cam/amp-resistant transforming units, exactly corresponding to the expected numbers which would be obtained by adding up the transducing units of the two individual aliquots. 48 cam/amp-resistant transductant colonies were picked from the plate where the mix of the two individual aliquots was used for infection and were analyzed by restriction digest. This showed that only the correct, SIP phage-producing vector combination (5 clones containing the fpep3_1B-IR3/pIG10.3-IMPp75 and 43 clones containing the fIMPp75-IR3/pIG10.3-pep10 combination; this represents a ratio of the two input vector combinations in the analyzed transductants of 1 : 8.6 (fpep3_1B-IR3/pIG10.3-IMPp75 : fIMPp75-IR3/pIG10.3-pep10), which is very similar to the 1 : 6.1 (fpep3_1B-IR3/pIG10.3-IMPp75 : fIMPp75-IR3/pIG10.3-pep10) ratio of double-resistant input phages in this experiment) occurred in all analyzed

transductants, verifying the presence of hetero-polyphages by ruling out the possibility of random co-infection and thus incorrect, random combination by two out of four possible monophage and/or homo-polyphage populations (fpep3_1B-IR3, pIG10.3-IMPp75, fIMPp75-IR3 and pIG10.3-pep10) each containing only one type of vector (phage or phagemid). Statistically, co-infection of the same bacterium by two separate phages was practically already excluded by the small numbers of infective phages containing at least one resistance marker (166 cam-resistant and 358 amp-resistant phages) which were used in the above experiment. Co-infection of the same bacterium (of a total of 10^7 bacteria) by one of the 166 cam-resistant phages and one of the 358 amp-resistant phages has a probability of 6×10^{-10} . Moreover, in this scenario incorrect combinations of individual phage and phagemid vectors (e.g. fpep3_1B-IR3/ pIG10.3-pep10 and fIMPp75-IR3/ pIG10.3-IMPp75) would be possible. The fact that only the correct vector combinations were found in all 48 transductants analyzed from this experiment further proved that co-transduction by hetero-polyphage and not random co-infection by homo-polyphage or monophage was the mechanism by which double-resistance was transduced.

2.3.: Construction of a phage-display system for Fab display

The constructs described in 3.2. can easily be modified to achieve the display of Fabs or a Fab library. In fpep3_1B-IR3seq, the jun part can be replaced by a VL-CL light chain repertoire having the appropriate 3'- and 5'-restriction sites similarly as described for pep_3-to construct fVL_1B-R408IR. In pIG10.3-IMPp75, the IMPp75 construct can be replaced by a repertoire of VH-CH1 heavy chains. After co-transformation of both repertoires into host cells and expression, a library of phage particles displaying Fab fragments is produced. Since fpep3_1B-IR3seq was set up for a SIP experiment by having just the C-terminal domain of gIII, the corresponding Fab-displaying phage particles are non-infectious. By adding a target molecule fused to an infectivity-mediating particle (N1-N2 domain of gIIIp), phages displaying target-binding Fab fragments can be selected by infecting host cells.

By replacing the truncated gIII part described above by a full-length copy of gIII, a Fab-display library of infectious phage particles is obtained, which can be screened against immobilized targets. Binding phages can be eluted and used to infect host cells.

By selecting for transductants conferring cam/amp-resistance to their host cells, polyphage infections can be selected in both cases. Thereby the information about both chains of the selected Fab fragments can be retrieved.

CLAIMS

1. A method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes, said method being characterized by screening or selecting for polyphage particles that contain said combination.
2. The method of claim 1, comprising the steps of
 - (a) providing a first library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to, and displayed at, the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;
 - (b) providing a second library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules of said second library are able to be packaged in a phage particle and carry or encode a second selectable and/or screenable property different from said first property;
 - (c) optionally, providing nucleic acid sequences encoding further members of a multimeric (poly)peptide complex;
 - (d) expressing members of said libraries of recombinant vectors mentioned in steps (a), (b), and optionally nucleic acid sequences mentioned in step (c), in appropriate host cells under appropriate conditions, so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;
 - (e) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;
 - (f) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said

- multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;
- (g) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (f);
- (h) identifying said combination of nucleic acid sequences.

3. The method of claim 1, comprising the steps of

(a) expressing in appropriate host cells under appropriate conditions

- (aa) genetically diverse nucleic acid sequences contained in a first library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to and displayed at the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;
- (ab) genetically diverse nucleic acid sequences contained in a second library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules are able to be packaged in a phage particle and carry or encode a second selectable and/or screenable property different from said first property;
- (ac) optionally, nucleic acid sequences encoding further members of a multimeric (poly)peptide complex,

so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;

- (b) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;
- (c) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;

- (d) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (c);
- (e) identifying said combination of nucleic acid sequences.
4. The method of anyone of claims 1 to 3, wherein the vectors of said first and said second library are a combination of a phage vector and a phagemid vector.
 5. The method of anyone of claims 1 to 3, wherein the vectors of said first and said second library are a combination of two phagemid vectors, said appropriate conditions comprising complementation of phage genes by a helper phage.
 6. The method of claim 5, wherein said two phagemid vectors are compatible.
 7. The method of claim 6, wherein said two phagemid vectors comprise a ColE1 and a p15A plasmid origin of replication.
 8. The method of claim 6, wherein said two phagemid vectors comprise a ColE1 and a mutated ColE1 origin.
 9. The method of anyone of claims 4 to 8, wherein said vectors and/or said helper phage comprise different phage origins of replication.
 10. The method of anyone of claim 4 to 9, wherein said phage vector, said phagemid vector(s) and/or said helper phage are interference resistant.
 11. The method of claim 10, wherein said phage vector, said phagemid vector(s) and/or said helper phage have mutations in the phage intergenic region(s), preferably in positions corresponding to position 5986 of f1, and/or in gene II, preferably in positions corresponding to position 143 of f1.
 12. The method of anyone of claims 10 to 11, wherein said phage vector, said phagemid vector(s) and/or said helper phage are, or are derived from, IR1 mutants such as R176, R382, R383, R407, R408, or from IR2 mutants.

13. The method of anyone of claims 4 to 11, wherein said vectors and/or said helper phage comprise hybrid nucleic acid sequences of f1, fd, and/or M13 derived sequences.
14. The method of anyone of claims 1 to 13, wherein said vector is, or is derived from, fpep3_1B-IR3seq with the sequence listed in Figure 4.
15. The method of claim 14, wherein said derivative is a phage comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
16. The method of claim 14, wherein said derivative is a phagemid comprising essentially the phage origin of replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
17. The method of claim 14, wherein said derivative is a helper phage comprising essentially the phage origin of replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
18. The method of anyone of claims 15 to 17, said derivatives comprise the combined fd/f1 origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.
19. The method of anyone of claims 1 to 18, wherein the gene VII contained in any of said vectors contains an amber mutation.
20. The method of claim 19, wherein said mutation is identical to those found in phage vectors R68 or R100.
21. The method of anyone of claims 1 to 20, wherein the gene IX contained in any of said vectors contains an amber mutation.

22. The method of claim 21, wherein said mutation is identical to that found in phage vector N18.
23. The method of anyone of claims 1 to 22, wherein said phage coat protein is gIIIp or gVIIIp.
24. The method of anyone of claims 1 to 23, wherein said phage particles are infectious by having a full-length copy of gIIIp.
25. The method of anyone of claims 1 to 24, wherein said phage particles are non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the displayed multimeric (poly)peptide complexes with a corresponding partner coupled to an infectivity-mediating particle.
26. The method of claim 25, wherein said truncated gIIIp comprises the C-terminal domain of gIIIp.
27. The method of claim 26, wherein said truncated gIIIp is derived from phage fCA55.
28. The method of anyone of claims 1 to 27, wherein said predetermined property is binding to a target.
29. The method of claim 28, wherein said multimeric (poly)peptide complex is a fragment of an immunoglobulin superfamily member.
30. The method of claim 29, wherein said multimeric (poly)peptide complex is a fragment of an immunoglobulin.
31. The method of claim 30, wherein said fragment is an Fv, dsFv or Fab fragment.
32. The method of anyone of claims 1 to 27, wherein said predetermined property is the activity to perform or to catalyze a reaction.

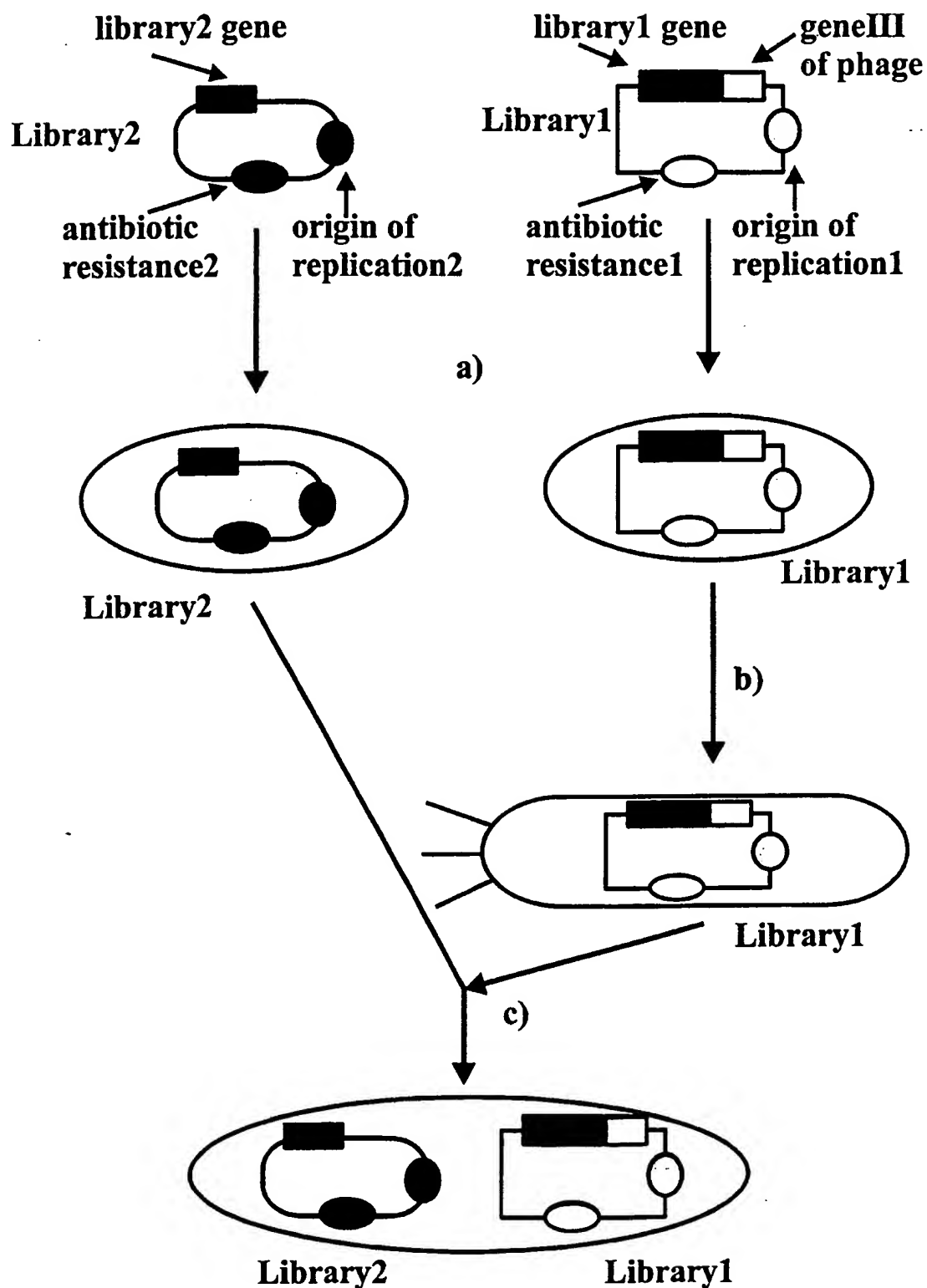
33. The method of claim 32, wherein said multimeric (poly)peptide complex is an enzyme.
34. The method of claim 33, wherein said multimeric (poly)peptide complex is a fragment of a catalytic antibody.
35. The method of claim 34, wherein said fragment is an Fv, dsFv or Fab fragment.
36. The method of anyone of claims 1 to 35, wherein said selectable and/or screenable property is the transactivation of transcription of a reporter gene such as beta-galactosidase, alkaline phosphatase or nutritional markers such as his3 and leu, or resistance genes giving resistance to an antibiotic such as ampicillin, chloramphenicol, kanamycin, zeocin, neomycin, tetracycline or streptomycin.
37. The method of anyone of claims 1 to 36, wherein said generation of said first and second screenable and/or selectable property is achieved after infection of appropriate host cells by said collection of phage particles.
38. The method of anyone of claims 1 to 37, wherein said identification of said nucleic acid sequences is effected by sequencing.
39. The method of anyone of claims 1 to 38, wherein said host cells are E.coli XL-1 Blue, K91 or derivatives thereof, TG1, XL1kann or TOP10F.
40. A polyphage particle which
- (a) contains
 - (i) a first recombinant vector molecule that comprises a nucleic acid sequence, which encodes a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, and that carries or encodes a first selectable and/or screenable property, and
 - (ii) a second recombinant vector molecule that comprises a nucleic acid sequence, which encodes a second member of a multimeric (poly)peptide complex, and that

carries or encodes a second selectable and/or screenable property different from said first property;
and (b) displays said multimeric (poly)peptide complex at its surface.

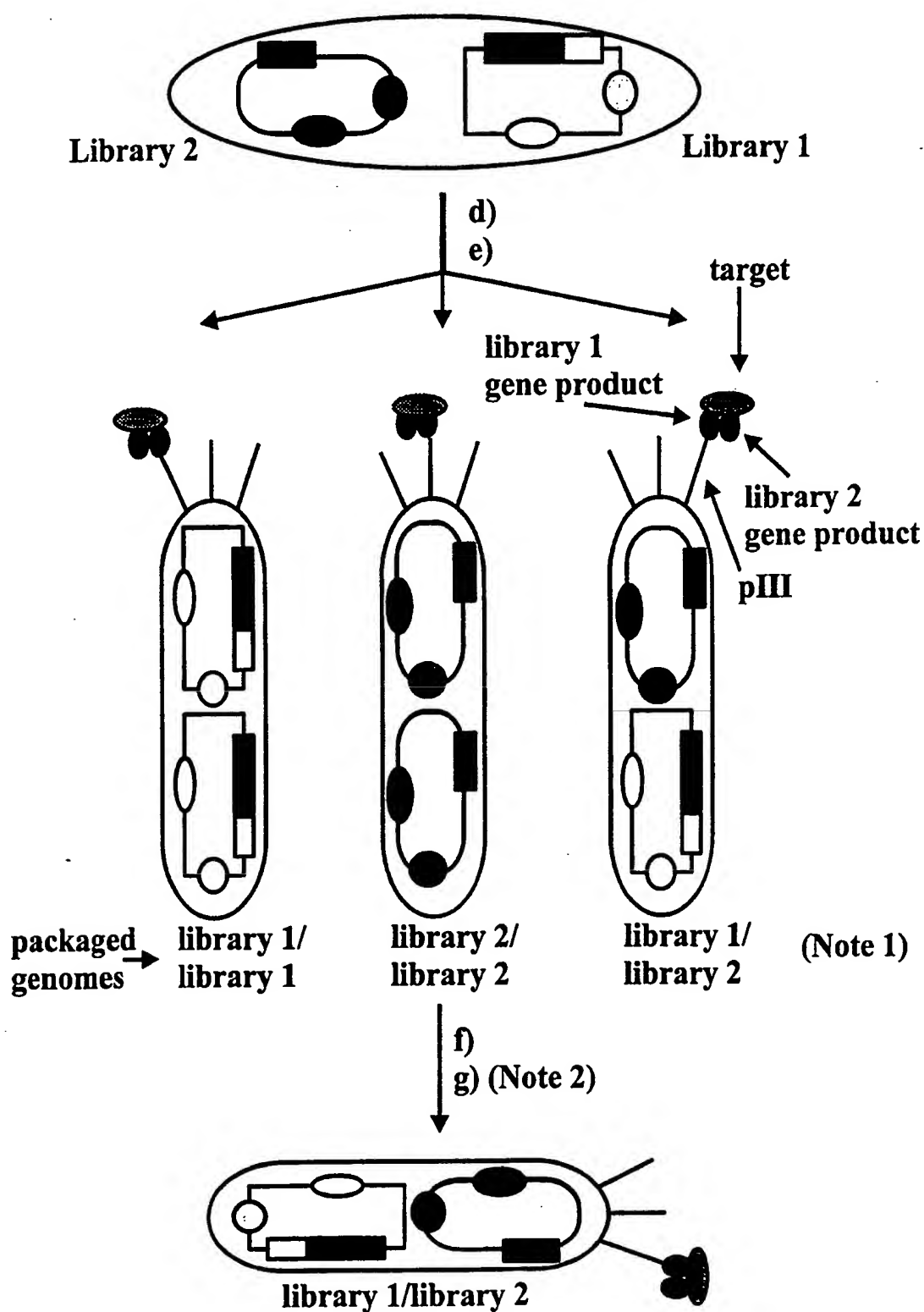
41. The polyphage particle according to claim 40 wherein said phage coat protein is the gIIIp.
42. The polyphage particle according to claim 41 wherein said particles is infectious by having a full-length copy of gIIIp present, either in said fusion protein, or in an additional wild-type copy.
43. The polyphage particle according to claim 41 wherein said particles is non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the displayed multimeric (poly)peptide complex with a corresponding partner coupled to an infectivity-mediating particle.
44. The phage vector fpep3_1B-IR3seq with the sequence listed in Figure 4.
45. A phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
46. A phagemid vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
47. A helper phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
48. A vector according to anyone of claims 45 to 47, wherein said derivatives comprise the combined fd/f1 origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.

49. The use according to any of the vectors of anyone of claims 44 to 48 in the generation of polyphage particles containing a combination of at least two different vectors.
50. The use according to claim 49, wherein said combination of different vectors comprises nucleic acid sequences encoding members of a multimeric (poly)peptide complex.
51. The use according to claim 50, wherein said combination of different vectors comprises nucleic acid sequences encoding interacting (poly)peptides/proteins.

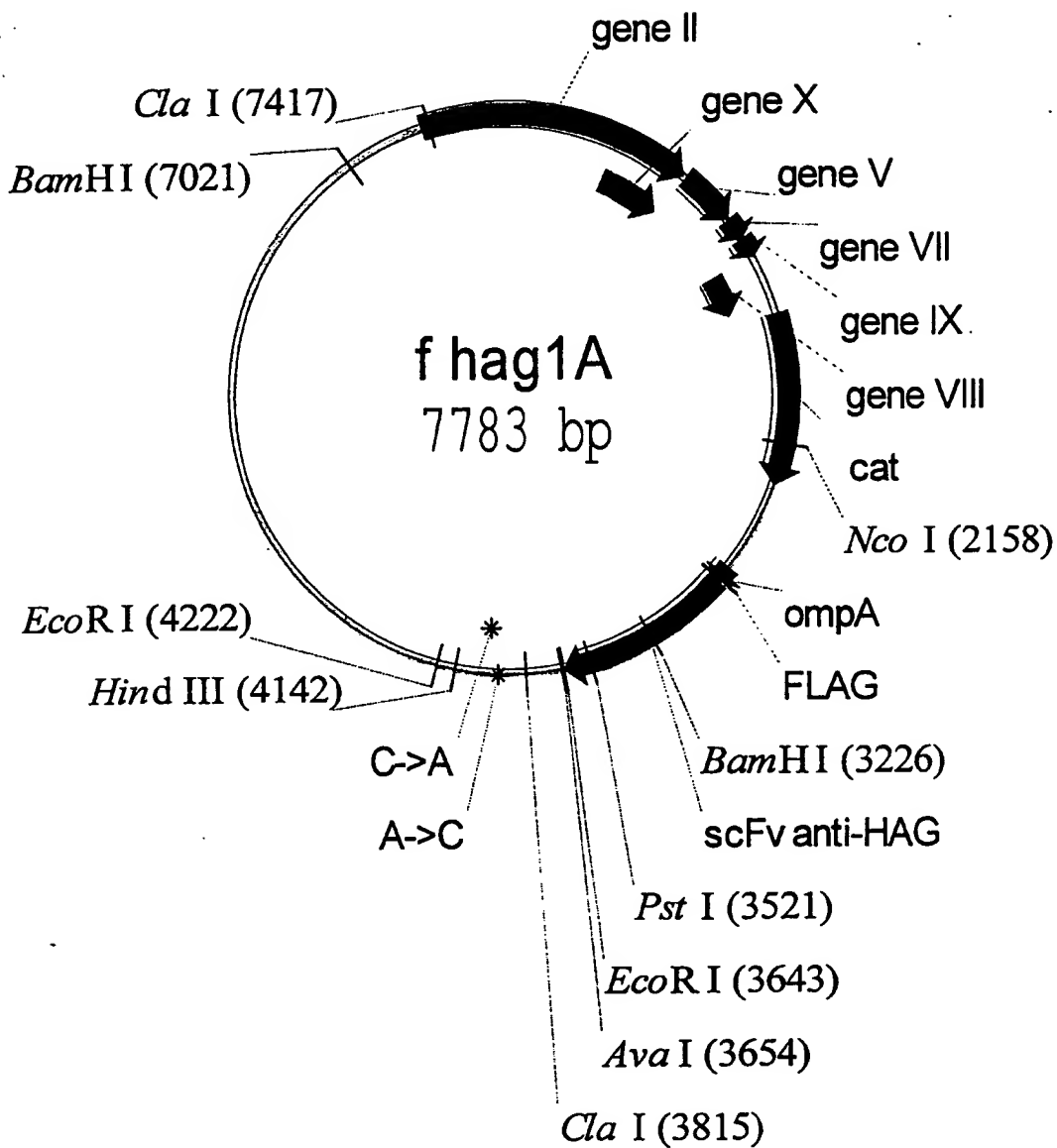
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Figure 1: General description of the polyphage principle

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Figure 1: General description of the phage principle (cont.)

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Figure 2

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1	AACGCTACTA	CCATTAGTAG	AATTGATGCC	ACCTTTTCAG	CTCGCGCCCC
	TTGCGATGAT	GGTAATCATC	TTAACTACGG	TGGAAAAGTC	GAGCGCGGGG
51	AAATGAAAAT	ATAGCTAAAC	AGGTTATTGA	CCATTTGCGA	AATGTATCTA
	TTTACTTTTA	TATCGATTGG	TCCAATAACT	GGTAAACGCT	TTACATAGAT
101	ATGGTCAAAC	TAAATCTACT	CGTTCGCAGA	ATTGGGAATC	AACTGTTACA
	TACCAGTTTG	ATTTAGATGA	GCAAGCGTCT	TAACCCTTAG	TTGACAATGT
151	TGGAATGAAA	CTTCCAGACA	CCGTACTTTA	GTTGCATATT	TAAAACATGT
	ACCTTACTTT	GAAGGTCTGT	GGCATGAAAT	CAACGTATAA	ATTTTGTACA
201	TGAACTACAG	CACCAGATTC	AGCAATTAAG	CTCTAAGCCA	TCCGCAAAAA
	ACTTGATGTC	GTGGTCTAAG	TCGTTAATTTC	GAGATTCGGT	AGGCGTTTTT
251	TGACCTCTTA	TCAAAAGGAG	CAATTAAAGG	TACTGTCTAA	TCCTGACCTG
	ACTGGAGAAT	AGTTTTCTCT	GTTAATTTCC	ATGACAGATT	AGGACTGGAC
301	TTGGAATTTG	CTTCCGGTCT	GGTTCGCTTT	GAGGCTCGAA	TTGAAACGCG
	AACCTTAAAC	GAAGGCCAGA	CCAAGCGAAA	CTCCGAGCTT	AACTTTGCGC
351	ATATTTGAAG	TCTTTCGGGC	TTCTCTTAA	TCTTTTTGAT	GCAATTCGCT
	TATAAACTTC	AGAAAGCCCG	AAGGAGAATT	AGAAAAACTA	CGTTAAGCGA
401	TTGCTTCTGA	CTATAATAGA	CAGGGTAAAG	ACCTGATTTT	TGATTTATGG
	AACGAAGACT	GATATTATCT	GTCCCATTTTC	TGGACTAAAA	ACTAAATACC
451	TCATTCTCGT	TTTCTGAACT	GTTTAAAGCA	TTTGAGGGGG	ATTCAATGAA
	AGTAAGAGCA	AAAGACTTGA	CAAATTTTCGT	AAACTCCCCC	TAAGTTACTT
501	TATTTATGAC	GATTCCGCAG	TATTGGACGC	TATCCAGTCT	AAACATTTTA
	ATAAATACTG	CTAAGGCGTC	ATAACCTGCG	ATAGGTCAGA	TTTGTAAAT
551	CAATTACCCC	CTCTGGCAAA	ACTTCCTTTG	CAAAAGCCTC	TCGCTATTTT
	GTTAATGGGG	GAGACCGTTT	TGAAGGAAAC	GTTTTTCGGAG	AGCGATAAAA
601	GGTTTCTATC	GTCGTCTGGT	TAATGAGGGT	TATGATAGTG	TTGCTCTTAC
	CCAAAGATAG	CAGCAGACCA	ATTACTCCCA	ATACTATCAC	AACGAGAATG
651	CATGCCTCGT	AATTCCTTTT	GGCGTTATGT	ATCTGCATTA	GTTGAGTGTG
	GTACGGAGCA	TTAAGGAAAA	CCGCAATACA	TAGACGTAAT	CAACTCACAC
701	GTATTCCTAA	ATCTCAATTG	ATGAATCTTT	CCACCTGTAA	TAATGTTGTT
	CATAAGGATT	TAGAGTTAAC	TACTTAGAAA	GGTGGACATT	ATTACAACAA
751	CCGTTAGTTC	GTTTTATTAA	CGTAGATTTT	TCCTCCCAAC	GTCCTGACTG
	GGCAATCAAG	CAAATAATT	GCATCTAAAA	AGGAGGGTTG	CAGGACTGAC
801	GTATAATGAG	CCAGTTCTTA	AAATCGCATA	AGGTAATTCA	AAATGATTAA
	CATATTACTC	GGTCAAGAAT	TTTAGCGTAT	TCCATTAAGT	TTTACTAATT

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851 AGTTGAAATT AAACCGTCTC AAGCGCAATT TACTACCCGT TCTGGTGTTT
 TCAACTTTAA TTTGGCAGAG TTCGCGTTAA ATGATGGGCA AGACCACAAA

 901 CTCGTCAGGG CAAGCCTTAT TCACTGAATG AGCAGCTTTG TTACGTTGAT
 GAGCAGTCCC GTTCGGAATA AGTGACTTAC TCGTCGAAAC AATGCAACTA

 951 TTGGGTAATG AATATCCGGT GCTTGTCAA G ATTACTCTCG ACGAAGGTCA
 AACCCATTAC TTATAGGCCA CGAACAGTTC TAATGAGAGC TGCTTCCAGT

 1001 GCCAGCGTAT GCGCCTGGTC TGTACACCGT GCATCTGTCC TCGTTCAAAG
 CGGTCGCATA CGCGGACCAG ACATGTGGCA CGTAGACAGG AGCAAGTTTC

 1051 TTGGTCAGTT CGGTTCTCTT ATGATTGACC GTCTGCGCCT CGTTCCGGCT
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 1101 AAGTAACATG GAGCAGGTCG CGGATTTCTGA CACAATTTAT CAGGCGATGA
 TTCATTGTAC CTCGTCCAGC GCCTAAAGCT GTGTTAAATA GTCCGCTACT

 1151 TACAAATCTC CGTTGTACTT TGTTCGCGC TTGGTATAAT CGCTGGGGGT
 ATGTTTAGAG GCAACATGAA ACAAAGCGCG AACCATATTA GCGACCCCCA

 1201 CAAAGATGAG TGTTTTAGTG TATTCCTTCG CCTCTTTCGT TTTAGGTTGG
 GTTCTACTC ACAAATCAC ATAAGAAAGC GGAGAAAGCA AAATCCAACC

 1251 TGCCTTCGTA GTGGCATTAC GTATTTTACC CGTTTAATGG AAACCTCCTC
 ACGGAAGCAT CACCGTAATG CATAAAATGG GCAAATTACC TTTGAAGGAG

 1301 ATGCGTAAGT CTTTAGTCCT CAAAGCCTCC GTAGCCGTTG CTACCCTCGT
 TACGCATTCA GAAATCAGGA GTTTCGGAGG CATCGGCAAC GATGGGAGCA

 1351 TCCGATGCTG TCTTTCGCTG CTGAGGGTGA CGATCCCGCA AAAGCGGCCT
 AGGCTACGAC AGAAAGCGAC GACTCCCACT GCTAGGGCGT TTTCGCCGGA

 1401 TTGACTCCCT GCAAGCCTCA GCGACCGAAT ATATCGGTTA TGCGTGGGCG
 AACTGAGGGA CGTTCGGAGT CGCTGGCTTA TATAGCCAAT ACGCACCCGC

 1451 ATGGTTGTTG TCATTGTCGG CGCAACTATC GGTATCAAGC TGTTTAAGAA
 TACCAACAAC AGTAACAGCC GCGTTGATAG CCATAGTTCG ACAAATTCTT

 1501 ATTCACCTCG AAAGCAAGCT GATAAAGGAG GTTCTCGAT CGAGACGTTN
 TAAGTGGAGC TTTCGTTCTGA CTATTTCTC CAAAGAGCTA GCTCTGCAAN

 1551 NNNGAGGTTC CAACTTTCAC CATAATGAAA TAAGATCACT ACCGGGCGTA
 NNNCTCCAAG GTTGAAAGTG GTATTACTTT ATTCTAGTGA TGGCCCGCAT

 1601 TTTTTTGAGT TATCGAGATT TTCAGGAGCT AAGGAAGCTA AAATGGAGAA
 AAAAACTCA ATAGCTCTAA AAGTCCTCGA TTCCTTCGAT TTTACCTCTT

 1651 AAAAATCACT GGATATACCA CCGTTGATAT ATCCCAATGG CATCGTAAAG
 TTTTATAGTGA CCTATATGGT GGCAACTATA TAGGGTTACC GTAGCATTTT

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TTGTAAAACT CCGTAAAGTC AGTCAACGAG TTACATGGAT ATTGGTCTGG

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1851 ATCCGGAGTT CCGTATGGCA ATGAAAGACG GTGAGCTGGT GATATGGGAT
TAGGCCTCAA GGCATACCGT TACTTTCTGC CACTCGACCA CTATACCTA

1901 AGTGTTCAAC CTTGTTACAC CGTTTTCCAT GAGCAAACCTG AAACGTTTTTC
TCACAAGTGG GAACAATGTG GCAAAAGGTA CTCGTTTGAC TTTGCAAAAG

1951 ATCGCTCTGG AGTGAATACC ACGACGATTT CCGGCAGTTT CTACACATAT
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2001 ATTCGCAAGA TGTGGCGTGT TACGGTGAAA ACCTGGCCTA TTTCCCTAAA
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2051 GGGTTTTATTG AGAATATGTT TTTCGTCTCA GCCAATCCCT GGGTGAGTTT
CCCAAATAAC TCTTATACAA AAAGCAGAGT CGGTTAGGGA CCCACTCAAA

2101 CACCAGTTTT GATTTAAACG TGGCCAATAT GGACAACTTC TTCGCCCCCG
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NcoI

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3351 GGTTTCTCCT TCTCCTCCTA CGGTATGTCC TGGGTTCGTC AGACCCCGGA
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3601 ACTGGGGTCA GGGTACCCTG GTTACCGTTT CAGCTTCCGG AGAATTCCAG
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AvaI

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EcoRI

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4751 TAAGCCTTCT ATTTCTGATT TGCTTGCTAT TGGTCGTGGT AATGATTCTT
ATTGGAAGA TAAAGACTAA ACGAACGATA ACCAGACCA TTACTAAGGA

4801 ACGACGAAAA TAAAAACGGT TTGCTTGTTT TTGATGAATG CGGTACTTGG
TGCTGCTTTT ATTTTGTCCA AACGAACAAG AACTACTTAC GCCATGAACC

4851 TTTAATACCC GTTCATGGAA TGACAAGGAA AGACAGCCGA TTATTGATTG
AAATTATGGG CAAGTACCTT ACTGTTCTT TCTGTCGGCT AATAACTAAC

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	TGATTTTATA	TAAACTTTTT	CAAAAGAGCG	CAAGAAACAG	AACGCTATCC
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	TAACAATTTA	CATTAATTAA	AACAAAAGAA	CTACAAACAA	AGTAGTAGAA
5651	CTTTTGCTCA	AGTAATTGAA	ATGAATAATT	CGCCTCTGCG	CGATTTTCGTG
	GAAAACGAGT	TCATTAACCT	TACTTATTAA	GCGGAGACGC	GCTAAAGCAC
5701	ACTTGGTATT	CAAAGCAAAC	AGGTGAATCT	GTTATTGTCT	CACCTGATGT
	TGAACCATAA	GTTTCGTTTG	TCCACTTAGA	CAATAACAGA	GTGGACTACA

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 ATTTCCATGT CACTGACATA TAAGGAGACT GCAATTCGGA CTTTTAAATG

5801 GCAATTTCTT TATCTCTGTT TTACGTGCTA ATAATTTTGA TATGGTTGGC
 CGTTAAAGAA ATAGAGACAA AATGCACGAT TATTAAACT ATACCAACCG

5851 TCAATTCCTT CCATAATTCA GAAATATAAC CCAAATAGTC AGGATTATAT
 AGTTAAGGAA GGTATTAAGT CTTTATATTG GGTTTATCAG TCCTAATATA

5901 TGATGAATTG CCATCATCTG ATATTCAGGA ATATGATGAT AATTCCGCTC
 ACTACTTAAC GGTAGTAGAC TATAAGTCCT TATACTACTA TTAAGGCGAG

5951 CTTCTGGTGG TTTCTTTGTT CCGCAAAATG ATAATGTTAC TCAAACATTT
 GAAGACCACC AAAGAAACAA GCGGTTTTAC TATTACAATG AGTTTGTAAG

6001 AAAATTAATA ACGTTCGCGC AAAGGATTTA ATAAGGGTTG TAGAATTGTT
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6051 TGTAAATCT AATACATCTA AATCCTCAA TGTATTATCT GTTGATGGTT
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6101 CTAACCTTATT AGTAGTTAGC GCCCTAAAG ATATTTTAGA TAACCTTCCG
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6151 CAATTTCTTT CTAAGTTTGA TTTGCCAACT GACCAGATAT TGATTGAAGG
 GTTAAAGAAA GATGACAACT AAACGGTTGA CTGGTCTATA ACTAAGTTCC

6201 ATTAATTTTC GAGGTTGAGC AAGGTGATGC TTTAGATTTT TCCTTTGCTG
 TAATTTAAAG CTCCAAGTCG TTCCACTACG AAATCTAAAA AGGAAACGAC

6251 CTGGCTCTCA GCGCGGCACT GTTGCTGGTG GTGTTAATAC TGACCGTCTA
 GACCGAGAGT CGCGCCGTGA CAACGACCAC CACAATTATG ACTGGCAGAT

6301 ACCTCTGTTT TATCTTCTGC GGGTGGTTTCG TTCGGTATTT TTAACGGCGA
 TGGAGACAAA ATAGAAGACG CCCACCAAGC AAGCCATAAA AATTGCCGCT

6351 TGTTTTAGGG CTATCAGTTC GCGCATTAAA GACTAATAGC CATTCAAAAA
 ACAAAATCCC GATAGTCAAG CGCGTAATTT CTGATTATCG GTAAGTTTTT

6401 TATTGTCTGT GCCTCGTATT CTTACGCTTT CAGGTCAGAA GGGTTCTATT
 ATAACAGACA CGGAGCATAA GAATGCGAAA GTCCAGTCTT CCCAAGATAA

6451 TCTGTTGGCC AGAATGTCCC TTTTATTACT GGTCGTGTAA CTGGTGAATC
 AGACAACCGG TCTTACAGGG AAAATAATGA CCAGCACATT GACCACTTAG

6501 TGCCAATGTA AATAATCCAT TTCAGACGGT TGAGCGTCAA AATGTTGGTA
 ACGGTTACAT TTATTAGGTA AAGTCTGCCA ACTCGCAGTT TTACAACCAT

6551 TTTCTATGAG TGTTTTTCCC GTTGCAATGG CTGGCGGTAA TATTGTTTTA
 AAAGATACTC AAAAAAGGG CAACGTTACC GACCGCCATT ATAACAAAAT

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6601 GATATAACCA GTAAGGCCGA TAGTTTGAGT TCTTCTACTC AGGCAAGTGA
CTATATTGGT CATTCCGGCT ATCAAACCTCA AGAAGATGAG TCCGTTCACT

6651 TGTTATTACT AATCAAAGAA GTATTGCGAC AACGGTTAAT TTGCGTGATG
ACAATAATGA TTAGTTTCTT CATAACGCTG TTGCCAATTA AACGCACTAC

6701 GTCAGACTCT TTTGCTCGGT GGCCTCACTG ATTACAAAAA CACTTCTCAA
CAGTCTGAGA AAACGAGCCA CCGGAGTGAC TAATGTTTTT GTGAAGAGTT

6751 GATTCTGGTG TGCCGTTTCT GTCTAAAATC CCTTTAATCG GCCTCCTGTT
CTAAGACCAC ACGGCAAGGA CAGATTTTAG GGAAATTAGC CGGAGGACAA

6801 TAGCTCCCGT TCTGATTCTA ACGAGGAAAG CACGTTGTAC GTGCTCGTCA
ATCGAGGGCA AGACTAAGAT TGCTCCTTTC GTGCAACATG CACGAGCAGT

6851 AAGCAACCAT AGTACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT
TTCGTTGGTA TCATGCGCGG GACATCGCCG CGTAATTCGC GCCGCCACA

6901 GGTGGTTACG CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG
CCACCAATGC GCGTCGCACT GCGGATGTGA ACGGTCGCGG GATCGCGGGC

6951 CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG CCACGTTCTC CGGCTTTCCC
GAGGAAAGCG AAAGAAGGGA AGGAAAGAGC GGTGCAAGAG GCCGAAAGGG

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7001 CGTCAAGCTC TAAATCGGGG GATCCCTTTA GGGTTCCGAT TTAGTGCTTT
GCAGTTCGAG ATTTAGCCCC CTAGGGAAAT CCCAAGGCTA AATCACGAAA

7051 ACGGCACCTC GACCTCCAAA AACTTGATTT GGGTGATGGT TCACGTAGTG
TGCCGTGGAG CTGGAGGTTT TTGAACTAAA CCCACTACCA AGTGCATCAC

7101 GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG
CCGGTAGCGG GACTATCTGC CAAAAAGCGG GAAACTGCAA CCTCAGGTGC

7151 TTCTTTAATA GTGGACTCTT GTTCCAAACT GGAACAACAC TCACAACTAA
AAGAAATTAT CACCTGAGAA CAAGGTTTGA CTTGTTGTG AGTGTTGATT

7201 CTCGGCCTAT TCTTTTGATT TATAAGGATT TTTGTCATTT TCTGCTTACT
GAGCCGGATA AGAAACTAA ATATTCCTAA AAACAGTAAA AGACGAATGA

7251 GGTTAAAAAA TAAGCTGATT TAACAAATAT TTAACGCGAA ATTTAACAAA
CCAATTTTTT ATTCGACTAA ATTGTTTATA AATTGCGCTT TAAATTGTTT

7301 ACATTAACGT TTACAATTTA AATATTTGCT TATACAATCA TCCTGTTTTT
TGTAATTGCA AATGTTAAAT TTATAAACGA ATATGTTAGT AGGACAAAAA

7351 GGGGCTTTTC TGATTATCAA CCGGGGTACA TATGATTGAC ATGCTAGTTT
CCCCGAAAAG ACTAATAGTT GGCCCCATGT ATACTAACTG TACGATCAAA

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7401 TACGATTACC GTTCATCGAT TCTCTTGTTT GCTCCAGACT TTCAGGTAAT  
ATGCTAATGG CAAGTAGCTA AGAGAACAAA CGAGGTCTGA AAGTCCATTA

7451 GACCTGATAG CCTTTGTAGA CCTCTCAAAA ATAGCTACCC TCTCCGGCAT  
CTGGACTATC GGAAACATCT GGAGAGTTTT TATCGATGGG AGAGGCCGTA

7501 GAATTTATCA GCTAGAACGG TTGAATATCA TATTGACGGT GATTTGACTG  
CTTAAATAGT CGATCTTGCC AACTTATAGT ATAAC TGCCA CTAAACTGAC

7551 TCTCCGGCCT TTCTCACCCG TTTGAATCTT TGCCTACTCA TTA CTCCGGC  
AGAGGCCGGA AAGAGTGGGC AACTTAGAA ACGGATGAGT AATGAGGCCG

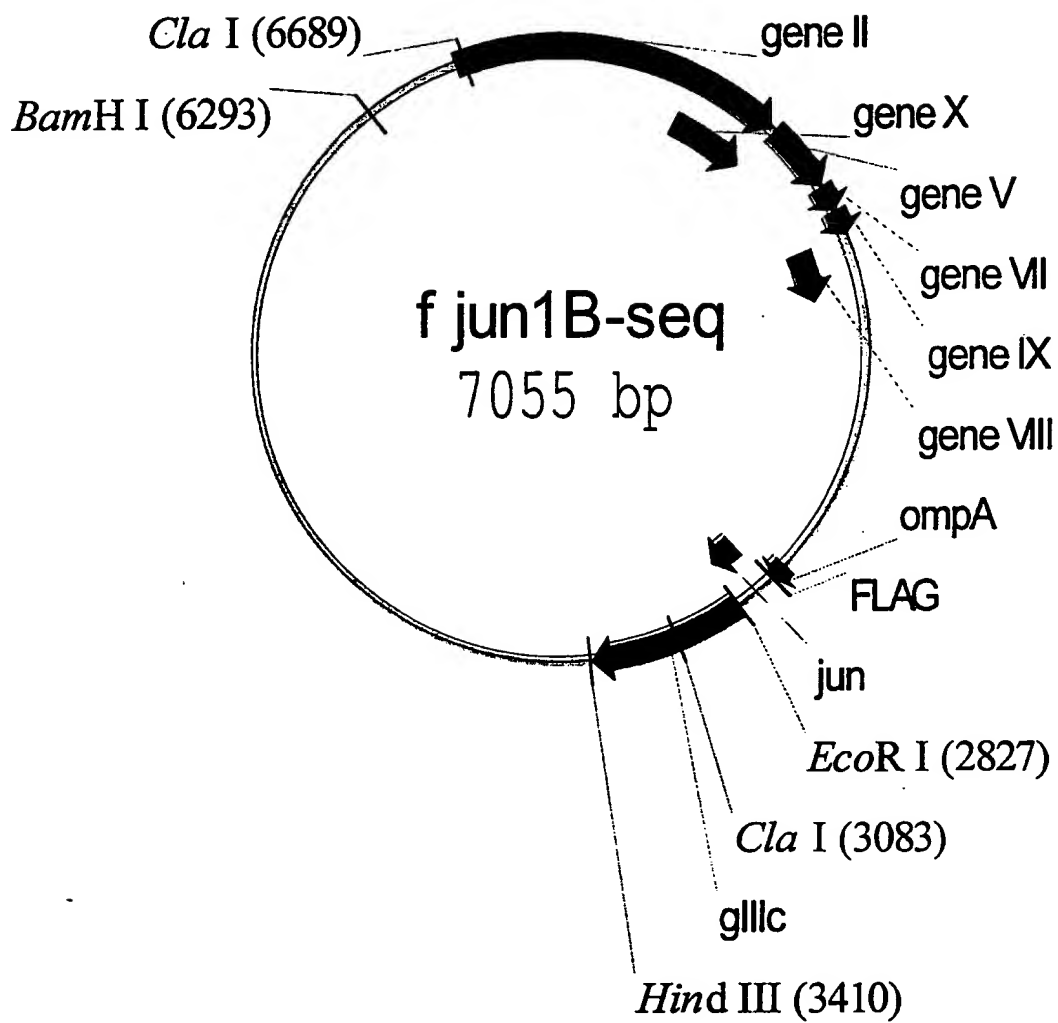
7601 ATTGCATTTA AAATATATGA GGGTTCTAAA AATTTTTATC CCTGCGTTGA  
TAACGTAAAT TTTATATACT CCAAGATTT TTAAAAATAG GGACGCAACT

7651 AATTAAGGCT TCACCAGCAA AAGTATTACA GGGTCATAAT GTTTTTGGTA  
TTAATTCCGA AGTGGTCGTT TTCATAATGT CCCAGTATTA CAAAAACCAT

7701 CAACCGATTT AGCTTTATGC TCTGAGGCTT TATTGCTTAA TTTTGCTAAC  
GTTGGCTAAA TCGAAATACG AGACTCCGAA ATAACGAATT AAAACGATTG

7751 TCTCTGCCTT GCTTGACGA TTTATTGGAT GTT  
AGAGACGGAA CGAACATGCT AAATAACCTA CAA

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**Figure 3**

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1 AACGCTACTA CCATTAGTAG AATTGATGCC ACCTTTTCAG CTCGCGCCCC  
 TTGCGATGAT GGTAATCATC TTAAC TACGG TGGAAAAGTC GAGCGCGGGG

51 AAATGAAAAT ATAGCTAAAC AGGTTATTGA CCATTTGCGA AATGTATCTA  
 TTTACTTTTA TATCGATTTG TCCAATAACT GGTAAACGCT TTACATAGAT

101 ATGGTCAAAC TAAATCTACT CGTTCGCAGA ATTGGGAATC AACTGTTACA  
 TACCAGTTTG ATTTAGATGA GCAAGCGTCT TAACCCTTAG TTGACAATGT

151 TGAATGAAA CTTCCAGACA CCGTACTTTA GTTGCAATATT TAAAACATGT  
 ACCTTACTTT GAAGGTCTGT GGCATGAAAT CAACGTATAA ATTTTGTACA

201 TGAAC TACAG CACCAGATTC AGCAATTAAG CTCTAAGCCA TCCGCAAAAA  
 ACTTGATGTC GTGGTCTAAG TCGTTAATTC GAGATTCGGT AGGCGTTTTT

251 TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTGTCTAA TCCTGACCTG  
 ACTGGAGAAT AGTTTTCTCTC GTTAATTTCC ATGACAGATT AGGACTGGAC

301 TTGGAATTTG CTTCCGGTCT GGTTTCGCTTT GAGGCTCGAA TTGAAACGCG  
 AACCTTAAAC GAAGGCCAGA CCAAGCGAAA CTCCGAGCTT AACTTTGCGC

351 ATATTTGAAG TCTTTTCGGC TTCCTCTTAA TCTTTTTGAT GCAATTCGCT  
 TATAAACTTC AGAAAGCCCC AAGGAGAATT AGAAAACTA CGTTAAGCGA

401 TTGCTTCTGA CTATAATAGA CAGGGTAAAG ACCTGATTTT TGATTTATGG  
 AACGAAGACT GATATTATCT GTCCCATTTT TGGACTAAAA ACTAAATACC

451 TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCAATGAA  
 AGTAAGAGCA AAAGACTTGA CAAATTTTCGT AAAC TCCCC TAAGTTACTT

501 TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA  
 ATAAATACTG CTAAGGCGTC ATAACCTGCG ATAGGTCAGA TTTGTAAAT

551 CAATTACCCC CTCTGGCAAA ACTTCCTTTG CAAAAGCCTC TCGCTATTTT  
 GTTAATGGGG GAGACCGTTT TGAAGGAAAC GTTTTCGGAG AGCGATAAAA

601 GGTTTCTATC GTCGTCTGGT TAATGAGGGT TATGATAGTG TTGCTCTTAC  
 CCAAAGATAG CAGCAGACCA ATTACTCCCA ATACTATCAC AACGAGAATG

651 CATGCCTCGT AATTCCTTTT GGCGTTATGT ATCTGCATTA GTTGAGTGTG  
 GTACGGAGCA TTAAGGAAAA CCGCAATACA TAGACGTAAT CACTCACAC

701 GTATTCCTAA ATCTCAATTG ATGAATCTTT CCACCTGTAA TAATGTTGTT  
 CATAAGGATT TAGAGTTAAC TACTTAGAAA GGTGGACATT ATTACAACA

751 CCGTTAGTTC GTTTTATTAA CGTAGATTTT TCCTCCCAAC GTCCTGACTG  
 GGCAATCAAG CAAAATAATT GCATCTAAAA AGGAGGGTTG CAGGACTGAC

801 GTATAATGAG CCAGTTCTTA AAATCGCATA AGGTAATTCA AAATGATTAA  
 CATATTACTC GGTCAAGAAT TTTAGCGTAT TCCATTAAGT TTTACTAATT



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|      |            |            |             |             |            |
|------|------------|------------|-------------|-------------|------------|
| 851  | AGTTGAAATT | AAACCGTCTC | AAGCGCAATT  | TACTACCCGT  | TCTGGTGTTC |
|      | TCAACTTTAA | TTTGGCAGAG | TTGCGTTAA   | ATGATGGGCA  | AGACCACAAA |
| 901  | CTCGTCAGGG | CAAGCCTTAT | TCACTGAATG  | AGCAGCTTTG  | TTACGTTGAT |
|      | GAGCAGTCCC | GTTCGGAATA | AGTGACTTAC  | TCGTCGAAAC  | AATGCAACTA |
| 951  | TTGGGTAATG | AATATCCGGT | GCTTGTCAAG  | ATTACTCTCG  | ACGAAGGTCA |
|      | AACCCATTAC | TTATAGGCCA | CGAACAGTTC  | TAATGAGAGC  | TGCTTCCAGT |
| 1001 | GCCAGCGTAT | GCGCCTGGTC | TGTACACCGT  | GCATCTGTCC  | TCGTTCAAAG |
|      | CGGTCGCATA | CGCGGACCAG | ACATGTGGCA  | CGTAGACAGG  | AGCAAGTTTC |
| 1051 | TTGGTCAGTT | CGGTTCTCTT | ATGATTGACC  | GTCTGCGCCT  | CGTTCCGGCT |
|      | AACCAGTCAA | GCCAAGAGAA | TACTAACTGG  | CAGACGCGGA  | GCAAGGCCGA |
| 1101 | AAGTAACATG | GAGCAGGTCG | CGGATTTCTGA | CACAATTTAT  | CAGGCGATGA |
|      | TTCATTGTAC | CTCGTCCAGC | GCCTAAAGCT  | GTGTTAAATA  | GTCCGCTACT |
| 1151 | TACAAATCTC | CGTTGTACTT | TGTTTTGCGC  | TTGGTATAAT  | CGCTGGGGGT |
|      | ATGTTTAGAG | GCAACATGAA | ACAAAGCGCG  | AACCATATTA  | GCGACCCCCA |
| 1201 | CAAAGATGAG | TGTTTTAGTG | TATTCTTTTCG | CCTCTTTTCGT | TTTAGGTTGG |
|      | GTTTCTACTC | ACAAAATCAC | ATAAGAAAGC  | GGAGAAAGCA  | AAATCCAACC |
| 1251 | TGCCTTCGTA | GTGGCATTAC | GTATTTTACC  | CGTTTAATGG  | AAACTTCCTC |
|      | ACGGAAGCAT | CACCGTAATG | CATAAAATGG  | GCAAATTACC  | TTTGAAGGAG |
| 1301 | ATGCGTAAGT | CTTTAGTCCT | CAAAGCCTCC  | GTAGCCGTTG  | CTACCCTCGT |
|      | TACGCATTCA | GAAATCAGGA | GTTTCGGAGG  | CATCGGCAAC  | GATGGGAGCA |
| 1351 | TCCGATGCTG | TCTTTCGCTG | CTGAGGGTGA  | CGATCCCGCA  | AAAGCGGCCT |
|      | AGGCTACGAC | AGAAAGCGAC | GACTCCCACT  | GCTAGGGCGT  | TTTCGCCGGA |
| 1401 | TTGACTCCCT | GCAAGCCTCA | GCGACCGAAT  | ATATCGGTTA  | TGCGTGGGCG |
|      | AACTGAGGGA | CGTTCGGAGT | CGCTGGCTTA  | TATAGCCAAT  | ACGCACCCGC |
| 1451 | ATGGTTGTTG | TCATTGTCGG | CGCAACTATC  | GGTATCAAGC  | TGTTTAAGAA |
|      | TACCAACAAC | AGTAACAGCC | GCGTTGATAG  | CCATAGTTTCG | ACAAATTCCT |
| 1501 | ATTCACCTCG | AAAGCAAGCT | GATAAAGGAG  | GTTTCTCGAT  | CGAGACGTTN |
|      | TAAGTGAGC  | TTTCGTTTCA | CTATTTCTCTC | CAAAGAGCTA  | GCTCTGCAAN |
| 1551 | NNNGAGGTTC | CAACTTTCAC | CATAATGAAA  | TAAGATCACT  | ACCGGGCGTA |
|      | NNNCTCCAAG | GTTGAAAGTG | GTATTACTTT  | ATTCTAGTGA  | TGGCCCCGAT |
| 1601 | TTTTTTGAGT | TATCGAGATT | TTCAGGAGCT  | AAGGAAGCTA  | AAATGGAGAA |
|      | AAAAAATCA  | ATAGCTCTAA | AAGTCCTCGA  | TTCCTTCGAT  | TTTACCTCTT |
| 1651 | AAAAATCACT | GGATATACCA | CCGTTGATAT  | ATCCCAATGG  | CATCGTAAAG |
|      | TTTTTAGTGA | CCTATATGGT | GGCAACTATA  | TAGGGTTACC  | GTAGCATTTT |

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|      |             |             |             |             |             |
|------|-------------|-------------|-------------|-------------|-------------|
| 1701 | AACATTTTGA  | GGCATTTTCAG | TCAGTTTGCTC | AATGTACCTA  | TAACCAGACC  |
|      | TTGTAAAACT  | CCGTAAAGTC  | AGTCAACGAG  | TTACATGGAT  | ATTGGTCTGG  |
| 1751 | G TTCAGCTGG | ATATTACGGC  | CTTTTAAAG   | ACCGTAAAGA  | AAAATAAGCA  |
|      | CAAGTCGACC  | TATAATGCCG  | GAAAAATTC   | TGGCATTCT   | TTTTATTCTG  |
| 1801 | CAAGTTTTAT  | CCGGCCTTTA  | TTCACATTCT  | TGCCCCCTG   | ATGAATGCTC  |
|      | GTTCAAAATA  | GGCCGGAAT   | AAGTGTAAGA  | ACGGGCGGAC  | TACTTACGAG  |
| 1851 | ATCCGGAGTT  | CCGTATGGCA  | ATGAAAGACG  | GTGAGCTGGT  | GATATGGGAT  |
|      | TAGGCCTCAA  | GGCATACCGT  | TACTTCTGTC  | CACTCGACCA  | CTATACCCTA  |
| 1901 | AGTGTTTACC  | CTTGTTACAC  | CGTTTTCCAT  | GAGCAAACCTG | AAACGTTTTTC |
|      | TCACAAGTGG  | GAACAATGTG  | GCAAAAAGTA  | CTCGTTTGAC  | TTTGCAAAAG  |
| 1951 | ATCGCTCTGG  | AGTGAATACC  | ACGACGATTT  | CCGGCAGTTT  | CTACACATAT  |
|      | TAGCGAGACC  | TCACTTATGG  | TGCTGCTAAA  | GGCCGTCAAA  | GATGTGTATA  |
| 2001 | ATTGCAAGA   | TGTGGCGTGT  | TACGGTGAAA  | ACCTGGCCTA  | TTTCCCTAAA  |
|      | TAAGCGTTCT  | ACACCGCACA  | ATGCCACTTT  | TGGACCGGAT  | AAAGGGATTT  |
| 2051 | GGGTTTATTG  | AGAATATGTT  | TTTCGTCTCA  | GCCAATCCCT  | GGGTGAGTTT  |
|      | CCCAAATAAC  | TCTTATACAA  | AAAGCAGAGT  | CGGTTAGGGA  | CCCACTCAAA  |
| 2101 | CACCAGTTTT  | GATTTAAACG  | TAGCCAATAT  | GGACAACTTC  | TTCGCCCCCG  |
|      | GTGGTCAAAA  | CTAAATTTGC  | ATCGGTTATA  | CCTGTTGAAG  | AAGCGGGGGC  |
| 2151 | TTTTCACTAT  | GGGCAAATAT  | TATACGCAAG  | GCGACAAGGT  | GCTGATGCCG  |
|      | AAAAGTGATA  | CCCGTTTATA  | ATATGCGTTC  | CGCTGTTCCA  | CGACTACGGC  |
| 2201 | CTGGCGATTG  | AGGTTTATCA  | TGCCGTTTGT  | GATGGCTTCC  | ATGTCGGCAG  |
|      | GACCGCTAAG  | TCCAAGTAGT  | ACGGCAAACA  | CTACCGAAGG  | TACAGCCGTC  |
| 2251 | AATGCTTAAT  | GAATTACAAC  | AGTACTGCGA  | TGAGTGGCAG  | GGCGGGGCGT  |
|      | TTACGAATTA  | CTTAATGTTG  | TCATGACGCT  | ACTCACCGTC  | CCGCCCCGCA  |
| 2301 | AATTTTTTTA  | AGGCAGTTAT  | TGGTGCCCTT  | AAACGCCTGG  | TGCTAGCCTG  |
|      | TTAAAAAAAT  | TCCGTCAATA  | ACCACGGGAA  | TTTGCGGACC  | ACGATCGGAC  |
| 2351 | AGGCCAGTTT  | GCTCAGGCTC  | TCCCCGTGGA  | GGTAATAATT  | GCTCGACCGA  |
|      | TCCGGTCAAA  | CGAGTCCGAG  | AGGGGCACCT  | CCATTATTAA  | CGAGCTGGCT  |
| 2401 | TAAAAGCGGC  | FTCCTGACAG  | GAGGCCGTTT  | TGTTTTGCAG  | CCCACCTCAA  |
|      | ATTTTCGCCG  | AAGGACTGTC  | CTCCGGCAAA  | ACAAAACGTC  | GGGTGGAGTT  |
| 2451 | CGCAATTAAT  | GTGAGTTAGC  | TCACTCATT   | GGCACCCAG   | GCTTTTACACT |
|      | GCGTTAATTA  | CACTCAATCG  | AGTGAGTAAT  | CCGTGGGGTC  | CGAAATGTGA  |
| 2501 | TTATGCTTCC  | GGCTCGTATG  | TTGTGTGGAA  | TTGTGAGCGG  | ATAACAATTT  |
|      | AATACGAAGG  | CCGAGCATAC  | AACACACCTT  | AACACTCGCC  | TATTGTTAAA  |

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2551 CACACAGGAA ACAGCTATGA CCATGATTAC GAATTTCTAG ATAACGAGGG  
GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAAAGATC TATTGCTCCC

2601 CAAAAAATGA AAAAGACAGC TATCGCGATT GCAGTGGCAC TGGCTGGTTT  
GTTTTTTACT TTTTCTGTCTG ATAGCGCTAA CGTCACCGTG ACCGACCAAA

2651 CGCTACCGTA GCGCAGGCCG ACTACAAAGA TGTCGACGCC GGTGGTCCGA  
GCGATGGCAT CCGTCCGGC TGATGTTTCT ACAGCTGCGG CCACCAGCCT

2701 TCGCCCGGCT AGAGGAAAAA GTGAAAACCT TGAAAGCGCA AAAC TCCGAG  
AGCGGGCCGA TCTCCTTTTT CACTTTTGGA ACTTTCGCGT TTTGAGGCTC

2751 CTGGCGTCCA CGGCCAACAT GCTCAGGGAA CAGGTGGCAC AGCTTAAACA  
GACCGCAGGT GCCGGTTGTA CGAGTCCCTT GTCCACCGTG TCGAATTTGT

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2801 GAAAGTCATG AACCACGGTG GTGCCGAATT CAATGCTGGC GGCGGCTCTG  
CTTTCAGTAC TTGGTGCCAC CACGGCTTAA GTTACGACCG CCGCCGAGAC

2851 GTGGTGGTTC TGGTGGCGGC TCTGAGGGTG GTGGCTCTGA GGGTGGCGGT  
CACCACCAAG ACCACCGCCG AGACTCCAC CACCGAGACT CCCACCGCCA

2901 TCTGAGGGTG GCGGCTCTGA GGGAGGCGGT TCCGGTGGTG GCTCTGGTTC  
AGACTCCAC CGCCGAGACT CCCTCCGCCA AGGCCACCAC CGAGACCAAG

2951 CGGTGATTTT GATTATGAAA AGATGGCAAA CGCTAATAAG GGGGCTATGA  
GCCACTAAAA CTAATACTTT TCTACCGTTT GCGATTATTC CCCC GATACT

3001 CCGAAAATGC CGATGAAAAC GCGCTACAGT CTGACGCTAA AGGCAAAC TT  
GGCTTTTACG GCTACTTTTG CGCGATGTCA GACTGCGATT TCCGTTTGAA

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3051 GATTCTGTCTG CTA CTGATTA CGGTGCTGCT ATCGATGGTT TCATTGGTGA  
CTAAGACAGC GATGACTAAT GCCACGACGA TAGCTACCAA AGTAACCACT

3101 CGTTTCCGGC CTTGCTAATG GTAATGGTGC TACTGGTGAT TTTGCTGGCT  
GCAAAGGCCG GAACGATTAC CATTACCACG ATGACCACTA AAACGACCGA

3151 CTAATTCCCA AATGGCTCAA GTCGGTGACG GTGATAATTC ACCTTTAATG  
GATTAAGGGT TTACCGAGTT CAGCCACTGC CACTATTAAG TGGAAATTAC

3201 AATAATTTCC GTCAATATTT ACCTTCCCTC CCTCAATCGG TTGAATGTCG  
TTATTAAAGG CAGTTATAAA TGGAAGGGAG GGAGTTAGCC AACTTACAGC

3251 CCCTTTTGTC TTTAGCGCTG GTAAACCATA TGAATTTTCT ATTGATTGTG  
GGGAAAACAG AAATCGCGAC CATTGTTGAT ACTTAAAAGA TAACTAACAC

3301 ACAAATAAAA CTTATTCCGT GGTGTCTTTG CGTTTCTTTT ATATGTTGCC  
TGTTTTATTT GAATAAGGCA CCACAGAAAC GCAAAGAAAA TATACAACGG

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3351 ACCTTTATGT ATGTATTTTC TACGTTTGCT AACATACTGC GTAATAAGGA  
TGGAAATACA TACATAAAAG ATGCAAACGA TTGTATGACG CATTATTCCT

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3401 GTCTTGATAA GCTTCGAGAA ATTCACCTCG AAAGCAAGCT GATAAACCGA  
CAGAACTATT CGAAGCTCTT TAAGTGGAGC TTTCGTTCTGA CTATTTGGCT

3451 TACAATTAAA GGCTCCTTTT GGAGCCTTTT TTTTGGAGA ATTAATTCAA  
ATGTTAATTT CCGAGGAAAA CCTCGGAAAA AAAAACCTCT TAATTAAGTT

3501 TCATGCCAGT TCTTTTGGGT ATTCCGTTAT TATTGCGTTT CCTCGGTTTC  
AGTACGGTCA AGAAAACCCA TAAGGCAATA ATAACGCAA GGAGCCAAAG

3551 CTTCTGGTAA CTTTGTTCTGG CTATCTGCTT ACTTTCCTTA AAAAGGGCTT  
GAAGACCATT GAAACAAGCC GATAGACGAA TGAAAGGAAT TTTTCCCGAA

3601 CGGTAAGATA GCTATTGCTA TTTCATTGTT TCTTGCTCTT ATTATTGGGC  
GCCATTCTAT CGATAACGAT AAAGTAACAA AGAACGAGAA TAATAACCCG

3651 TTAACCAAT TCTTGTGGGT TATCTCTCTG ATATTAGCGC ACAATTACCC  
AATTGAGTTA AGAACACCCA ATAGAGAGAC TATAATCGCG TGTTAATGGG

3701 TCTGATTTTG TTCAGGGCGT TCAGTTAATT CTCCCGTCTA ATGCGCTTCC  
AGACTAAAAC AAGTCCCGCA AGTCAATTAA GAGGGCAGAT TACGCGAAGG

3751 CTGTTTTTAT GTTATTCTCT CTGTAAAGGC TGCTATTTTC ATTTTGGACG  
GACAAAAATA CAATAAGAGA GACATTTCG ACGATAAAAG TAAAACTGC

3801 TTAAACAAAA AATCGTTTCT TATTTGGATT GGGATAAATA AATATGGCTG  
AATTTGTTTT TTAGCAAAGA ATAAACCTAA CCCTATTTAT TTATACCGAC

3851 TTTATTTTGT AACTGGCAAA TTAGGCTCTG GAAAGACGCT CGTTAGCGTT  
AAATAAAACA TTGACCGTTT AATCCGAGAC CTTTCTGCGA GCAATCGCAA

3901 GGTAAGATTC AGGATAAAAT TGTAGCTGGG TGCAAAATAG CAACTAATCT  
CCATTCTAAG TCCTATTTTA ACATCGACCC ACGTTTTATC GTTGATTAGA

3951 TGATTTAAGG CTTCAAAACC TCCCGCAAGT CGGGAGGTTC GCTAAAACGC  
ACTAAATTCC GAAGTTTTGG AGGGCGTTCA GCCCTCCAAG CGATTTTGCG

4001 CTCGCGTTCT TAGAATACCG GATAAGCCTT CTATTTCTGA TTTGCTTGCT  
GAGCGCAAGA ATCTTATGGC CTATTCGGAA GATAAAGACT AAACGAACGA

4051 ATTGGTCGTG GTAATGATTC CTACGACGAA AATAAAAACG GTTTGCTTGT  
TAACCAGCAC CATTACTAAG GATGCTGCTT TTATTTTGC CAAACGAACA

4101 TCTTGATGAA TGCGGTACTT GGTTTAATAC CCGTTCATGG AATGACAAGG  
AGAATACTT ACGCCATGAA CCAAATTATG GGCAAGTACC TTAAGTTCCT

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4151 AAAGACAGCC GATTATTGAT TGGTTTCTTC ATGCTCGTAA ATTGGGATGG  
TTTCTGTCCG CTAATAACTA ACCAAAGAAG TACGAGCATT TAACCCTACC

4201 GATATTATTT TTCTTGTTCA GGATTTATCT ATTGTTGATA AACAGGCGCG  
CTATAATAAA AAGAACAAGT CCTAAATAGA TAACAACTAT TTGTCCGCGC

4251 TTCTGCATTA GCTGAACACG TTGTTTATTG TCGCCGTCTG GACAGAATTA  
AAGACGTAAT CGACTTGTGC AACAAATAAC AGCGGCAGAC CTGTCTTAAT

4301 CTTTACCCTT TGTCGGCACT TTATATTCTC TTGTTACTGG CTCAAAAATG  
GAAATGGGAA ACAGCCGTGA AATATAAGAG AACAAATGACC GAGTTTTTAC

4351 CCTCTGCCTA AATTACATGT TGGTGTGTGT AAATATGGTG ATTCTCAATT  
GGAGACGGAT TTAATGTACA ACCACAACAA TTTATACCAC TAAGAGTTAA

4401 AAGCCCTACT GTTGAGCGTT GGCTTTATAC TGGTAAGAAT TTATATAACG  
TTCGGGATGA CAACTCGCAA CCGAAATATG ACCATTCTTA AATATATTGC

4451 CATATGACAC TAAACAGGCT TTTTCCAGTA ATTATGATTC AGGTGTTTAT  
GTATACTGTG ATTTGTCCGA AAAAGGTCAT TAATACTAAG TCCACAAATA

4501 TCATATTTAA CCCCTTATTT ATCACACGGT CGGTATTTCA AACCATTAAA  
AGTATAAATT GGGGAATAAA TAGTGTGCCA GCCATAAAGT TTGGTAATTT

4551 TTTAGGTCAG AAGATGAAAT TAACTAAAAT ATATTTGAAA AAGTTTTCTC  
AAATCCAGTC TTCTACTTTA ATTGATTTTA TATAAACTTT TCAAAAGAG

4601 GCGTTCTTTG TCTTGCGATA GGATTTGCAT CAGCATTTAC ATATAGTTAT  
CGCAAGAAAC AGAACGCTAT CCTAAACGTA GTCGTAAATG TATATCAATA

4651 ATAACCCAAC CTAAGCCGGA GGTAAAAAAG GTAGTCTCTC AGACCTATGA  
TATTGGGTTG GATTCGGCCT CCAATTTTTC CATCAGAGAG TCTGGATACT

4701 TTTTGATAAA TTCACTATTG ACTCTTCTCA GCGTCTTAAT CTAAGCTATC  
AAAACATTTT AAGTGATAAC TGAGAAGAGT CGCAGAATTA GATTCGATAG

4751 GCTATGTTTT CAAGGATTCT AAGGGAAAAAT TAATTAATAG CGACGATTTA  
CGATACAAAA GTTCCTAAGA TTCCCTTTTA ATTAATTATC GCTGCTAAAT

4801 CAGAAGCAAG GTTATTCCAT CACATATATT GATTTATGTA CTGTTTCAAT  
GTCTTCGTTT CAATAAGGTA GTGTATATAA CTAAATACAT GACAAAGTTA

4851 TAAAAAAGGT AATTCAAATG AAATTGTTAA ATGTAATTAA TTTTGTTTTT  
ATTTTTTCCA TTAAGTTTAC TTTAACAATT TACATTAATT AAAACAAAAG

4901 TTGATGTTTG TTTCATCATC TTCTTTTGCT CAAGTAATTG AAATGAATAA  
AACTACAAAC AAAGTAGTAG AAGAAAACGA GTTCATTAAC TTTACTTATT

4951 TTCGCCTCTG CGCGATTTCTG TGAATTGGTA TTCAAAGCAA ACAGGTGAAT  
AAGCGGAGAC GCGCTAAAGC ACTGAACCAT AAGTTTCGTT TGTCCACTTA

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5001 CTGTTATTGT CTCACCTGAT GTTAAAGGTA CAGTGACTGT ATATTCCTCT  
 GACAATAACA GAGTGGACTA CAATTTCCAT GTCACGACA TATAAGGAGA  
 5051 GACGTTAAGC CTGAAAATTT ACGCAATTTT TTTATCTCTG TTTTACGTGC  
 CTGCAATTCG GACTTTTAAA TCGTTTAAAG AAATAGAGAC AAAATGCACG  
 5101 TAATAATTTT GATATGGTTG GCTCAATTCC TTCCATAATT CAGAAATATA  
 ATTATTAAAA CTATACCAAC CGAGTTAAGG AAGGTATTAA GTCTTTATAT  
 5151 ACCCAAATAG TCAGGATTAT ATTGATGAAT TGCCATCATC TGATATTCAG  
 TGGGTTTATC AGTCCTAATA TAACTACTTA ACGGTAGTAG ACTATAAGTC  
 5201 GAATATGATG ATAATTCGCG TCCTTCTGGT GGTTTCTTTG TTCCGCAAAA  
 CTTATACTAC TATTAAGGCG AGGAAGACCA CCAAAGAAAC AAGGCGTTTT  
 5251 TGATAATGTT ACTCAAACAT TTAAAATTAA TAACGTTTCG GCAAAGGATT  
 ACTATTACAA TGAGTTTGTA AATTTTAATT ATTGCAAGCG CGTTTCCTAA  
 5301 TAATAAGGGT TGTAGAATTG TTTGTAAAT CTAATACATC TAAATCCTCA  
 ATTATTCCCA ACATCTTAAC AAACAATTTA GATTATGTAG ATTTAGGAGT  
 5351 AATGTATTAT CTGTTGATGG TTCTAACTTA TTAGTAGTTA GCGCCCCTAA  
 TTACATAATA GACAACTACC AAGATTGAAT AATCATCAAT CGCGGGGATT  
 5401 AGATATTTTA GATAACCTTC CGCAATTTCT TTCTACTGTT GATTTGCCAA  
 TCTATAAAAT CTATTGGAAG GCGTTAAAGA AAGATGACAA CTAAACGGTT  
 5451 CTGACCAGAT ATTGATTGAA GGATTAATTT TCGAGGTTCA GCAAGGTGAT  
 GACTGGTCTA TAACTAACTT CCTAATTAAA AGCTCCAAGT CGTTCCACTA  
 5501 GCTTTAGATT TTTCCTTTGC TGCTGGCTCT CAGCGCGGCA CTGTTGCTGG  
 CGAAATCTAA AAAGGAAACG ACGACCGAGA GTCGCGCCGT GACAACGACC  
 5551 TGGTGTTAAT ACTGACCGTC TAACCTCTGT TTTATCTTCT GCGGGTGGTT  
 ACCACAATTA TGACTGGCAG ATTGGAGACA AAATAGAAGA CGCCACCAA  
 5601 CGTTCGGTAT TTTTAACGGC GATGTTTTAG GGCTATCAGT TCGCGCATT  
 GCAAGCCATA AAAATTGCCG CTACAAAATC CCGATAGTCA AGCGCGTAAT  
 5651 AAGACTAATA GCCATTCAAA AATATTGTCT GTGCCTCGTA TTCTTACGCT  
 TTCTGATTAT CGGTAAGTTT TTATAACAGA CACGGAGCAT AAGAATGCCA  
 5701 TTCAGGTCAG AAGGGTTCTA TTTCTGTTGG CCAGAATGTC CCTTTTATTA  
 AAGTCCAGTC TTCCAAGAT AAAGACAACC GGTCTTACAG GGAAATAAT  
 5751 CTGGTCGTGT AACTGGTGAA TCTGCCAATG TAAATAATCC ATTTACAGCG  
 GACCAGACA TTGACCACTT AGACGGTTAC ATTTATTAGG TAAAGTCTGC  
 5801 GTTGAGCGTC AAAATGTTGG TATTTCTATG AGTGTTTTTC CCGTTGCAAT  
 CAACTCGCAG TTTTACAACC ATAAAGATAC TCACAAAAG GGCAACGTTA

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|      |             |            |            |            |             |
|------|-------------|------------|------------|------------|-------------|
| 5851 | GGCTGGCGGT  | AATATTGTTT | TAGATATAAC | CAGTAAGGCC | GATAGTTTGA  |
|      | CCGACCGCCA  | TTATAACAAA | ATCTATATTG | GTCATTCCGG | CTATCAAAC   |
| 5901 | GTTCTTCTAC  | TCAGGCAAGT | GATGTTATTA | CTAATCAAAG | AAGTATTGCG  |
|      | CAAGAAGATG  | AGTCCGTTCA | CTACAATAAT | GATTAGTTTC | TTCATAACGC  |
| 5951 | ACAACGGTTA  | ATTTGCGTGA | TGGTCAGACT | CTTTTGCTCG | GTGGCCTCAC  |
|      | TGTTGCCAAT  | TAAACGCACT | ACCAGTCTGA | GAAAACGAGC | CACCGGAGTG  |
| 6001 | TGATTACAAA  | AACACTTCTC | AAGATTCTGG | TGTGCCGTTT | CTGTCTAAAA  |
|      | ACTAATGTTT  | TTGTGAAGAG | TTCTAAGACC | ACACGGCAAG | GACAGATTTT  |
| 6051 | TCCCTTTAAT  | CGGCCTCCTG | TTTAGCTCCC | GTTCTGATTC | TAACGAGGAA  |
|      | AGGGAAATTA  | GCCGGAGGAC | AAATCGAGGG | CAAGACTAAG | ATTGCTCCTT  |
| 6101 | AGCACGTTGT  | ACGTGCTCGT | CAAAGCAACC | ATAGTACGCG | CCCTGTAGCG  |
|      | TCGTGCAACA  | TGCACGAGCA | GTTTCGTTGG | TATCATGCGC | GGGACATCGC  |
| 6151 | GCGCATTAAAG | CGCGGCGGGT | GTGGTGGTTA | CGCGCAGCGT | GACCGCTACA  |
|      | CGCGTAATTC  | GCGCCGCCCA | CACCACCAAT | GCGCGTCGCA | CTGGCGATGT  |
| 6201 | CTTGCCAGCG  | CCCTAGCGCC | CGCTCCTTTC | GCTTTCTTCC | CTTCCTTTCT  |
|      | GAACGGTCGC  | GGGATCGCGG | GCGAGGAAAG | CGAAAGAAGG | GAAGGAAAGA  |
|      |             |            |            | BamHI      |             |
|      |             |            |            | ~~~~~      |             |
| 6251 | CGCCACGTTT  | TCCGGCTTTC | CCCGTCAAGC | TCTAAATCGG | GGGATCCCTT  |
|      | GCGGTGCAAG  | AGGCCGAAAG | GGGCAGTTTC | AGATTTAGCC | CCCTAGGGAA  |
| 6301 | TAGGGTTCCG  | ATTTAGTGCT | TTACGGCACC | TCGACCTCCA | AAAACCTTGAT |
|      | ATCCCAAGGC  | TAAATCACGA | AATGCCGTGG | AGCTGGAGGT | TTTGAACATA  |
| 6351 | TTGGGTGATG  | GTTACGCTAG | TGGGCCATCG | CCCTGATAGA | CGGTTTTTTCG |
|      | AACCCACTAC  | CAAGTGCAAT | ACCCGGTAGC | GGGACTATCT | GCCAAAAAGC  |
| 6401 | CCCTTTGACG  | TTGGAGTCCA | CGTTCTTTAA | TAGTGGACTC | TTGTTCCAAA  |
|      | GGGAAACTGC  | AACCTCAGGT | GCAAGAAATT | ATCACCTGAG | AACAAGGTTT  |
| 6451 | CTGGAACAAC  | ACTCACAAC  | AACTCGGCCT | ATTCTTTTGA | TTTATAAGGA  |
|      | GACCTTGTTG  | TGAGTGTTGA | TTGAGCCGGA | TAAGAAAAC  | AAATATTCCT  |
| 6501 | TTTTTGTCAT  | TTTCTGCTTA | CTGGTTAAAA | AATAAGCTGA | TTTAACAAAT  |
|      | AAAAACAGTA  | AAAGACGAAT | GACCAATTTT | TTATTCGACT | AAATTGTTTA  |
| 6551 | ATTTAACGCG  | AAATTTAACA | AAACATTAAC | GTTTACAATT | TAAATATTTG  |
|      | TAAATTGCGC  | TTTAAATTGT | TTTGTAATTG | CAAATGTTAA | ATTTATAAAC  |
| 6601 | CTTATACAAT  | CATCCTGTTT | TTGGGGCTTT | TCTGATTATC | AACCGGGGTA  |
|      | GAATATGTTA  | GTAGGACAAA | AACCCCGAAA | AGACTAATAG | TTGGCCCCAT  |

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6651 CATATGATTG ACATGCTAGT TTTACGATTA CCGTTCATCG ATTCTCTTGT
GTATACTAAC TGTACGATCA AAATGCTAAT GGCAAGTAGC TAAGAGAACA

6701 TTGCTCCAGA CTTTCAGGTA ATGACCTGAT AGCCTTTGTA GACCTCTCAA
AACGAGGTCT GAAAGTCCAT TACTGGACTA TCGGAAACAT CTGGAGAGTT

6751 AAATAGCTAC CCTCTCCGGC ATGAATTTAT CAGCTAGAAC GGTGAATAT
TTTATCGATG GGAGAGGCCG TACTTAAATA GTCGATCTTG CCAACTTATA

6801 CATATTGACG GTGATTTGAC TGTCTCCGGC CTTTCTCACC CGTTTGAATC
GTATAACTGC CACTAAACTG ACAGAGGCCG GAAAGAGTGG GCAAACCTAG

6851 TTTGCCTACT CATTACTCCG GCATTGCATT TAAAATATAT GAGGGTTCTA
AAACGGATGA GTAATGAGGC CGTAACGTAA ATTTTATATA CTCCCAAGAT

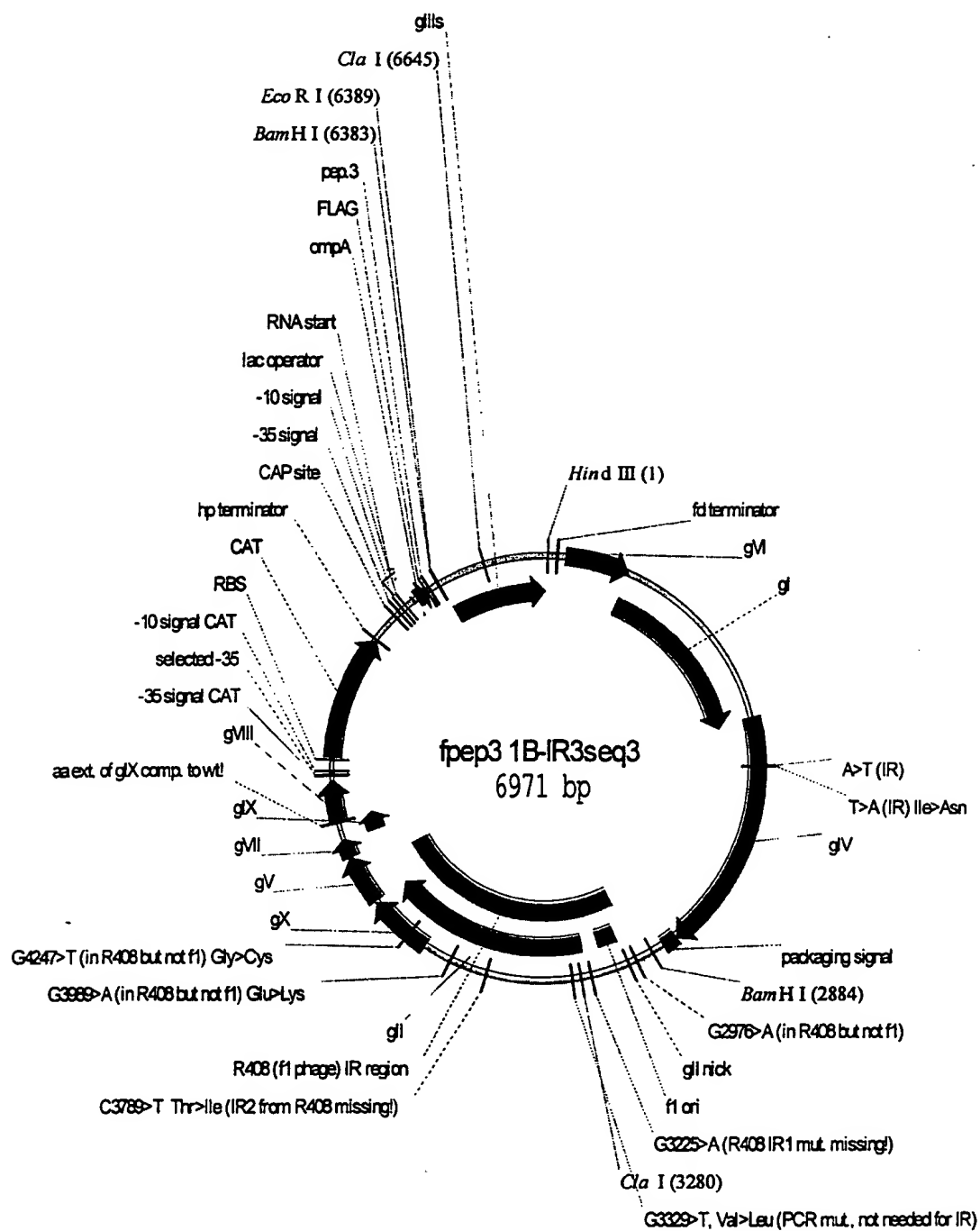
6901 AAAATTTTTA TCCCTGCGTT GAAATTAAGG CTTCACCAGC AAAAGTATTA
TTTTAAAAAT AGGGACGCAA CTTTAATTCC GAAGTGGTCG TTTTCATAAT

6951 CAGGGTCATA ATGTTTTTGG TACAACCGAT TTAGCTTTAT GCTCTGAGGC
GTCCCAGTAT TACAAAAACC ATGTTGGCTA AATCGAAATA CGGAGACTCCG

7001 TTTATTGCTT AATTTTGCTA ACTCTCTGCC TTGCTTGAC GATTTATTGG
AAATAACGAA TTAAAACGAT TGAGAGACGG AACGAACATG CTAATAAACC

7051 ATGTT
TACAA

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Figure 4

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1  AGCTTCGAGA AATTCACCTC GAAAGCAAGC TGATAAACCG ATACAATTAA
   TCGAAGCTCT TTAAGTGGAG CTTTCGTTCG ACTATTTGGC TATGTTAATT

51  AGGCTCCTTT TGGAGCCTTT TTTTTTGGAG AATTAATTCA ATCATGCCAG
   TCCGAGGAAA ACCTCGGAAA AAAAAACCTC TTAATTAAGT TAGTACGGTC

101 TTCTTTTGGG TATTCCGTTA TTATTGCGTT TCCTCGGTTT CCTTCTGGTA
   AAGAAAACCC ATAAGGCAAT AATAACGCAA AGGAGCCAAA GGAAGACCAT

151 ACTTTGTTCG GCTATCTGCT TACTTTCCTT AAAAAGGGCT TCGGTAAGAT
   TGAAACAAGC CGATAGACGA ATGAAAGGAA TTTTCCCGA AGCCATTCTA

201 AGCTATTGCT ATTTCATTGT TTCCTGCTCT TATTATTGGG CTTAACTCAA
   TCGATAACGA TAAAGTAACA AAGAACGAGA ATAATAACCC GAATTGAGTT

251 TTCTTGTGGG TTATCTCTCT GATATTAGCG CACAATTACC CTCTGATTTT
   AAGAACACCC AATAGAGAGA CTATAATCGC GTGTTAATGG GAGACTAAAA

301 GTTCAGGGCG TTCAGTTAAT TCTCCCGTCT AATGCGCTTC CCTGTTTTTA
   CAAGTCCCGC AAGTCAATTA AGAGGGCAGA TTACGCGAAG GGACAAAAAT

351 TGTTATTCTC TCTGTAAAGG CTGCTATTTT CATTTTGTAC GTTAAACAAA
   ACAATAAGAG AGACATTTCG GACGATAAAA GTAAAACTG CAATTTGTTT

401 AAATCGTTTC TTATTTGGAT TGGGATAAAT AAATATGGCT GTTTATTTTG
   TTTAGCAAAG AATAAACCTA ACCCTATTTA TTTATACCGA CAAATAAAAC

451 TAACTGGCAA ATTAGGCTCT GGAAAGACGC TCGTTAGCGT TGGTAAGATT
   ATTGACCGTT TAATCCGAGA CCTTCTGCG AGCAATCGCA ACCATTCTAA

501 CAGGATAAAA TTGTAGCTGG GTGCAAAATA GCAACTAATC TTGATTTAAG
   GTCCTATTTT AACATCGACC CACGTTTTAT CGTTGATTAG AACTAAATTC

551 GCTTCAAAAC CTCCGCAAG TCGGGAGGTT CGCTAAAACG CCTCGCGTTC
   CGAAGTTTTG GAGGGCGTTC AGCCCTCCAA GCGATTTTGC GGAGCGCAAG

601 TTAGAATACC GGATAAGCCT TCTATTTCTG ATTTGCTTGC TATTGGTCGT
   AATCTTATGG CCTATTCGGA AGATAAAGAC TAAACGAACG ATAACCAGCA

651 GGTAATGATT CCTACGACGA AAATAAAAAC GGTTTGCTTG TTCTTGATGA
   CCATTACTAA GGATGCTGCT TTTATTTTGT CCAAACGAAC AAGAACTACT

701 ATGCGGTACT TGGTTTAATA CCCGTTTCATG GAATGACAAG GAAAGACAGC
   TACGCCATGA ACCAAATTAT GGGCAAGTAC CTTACTGTTC CTTTCTGTGC

751 CGATTATTGA TTGGTTTCTT CATGCTCGTA AATTGGGATG GGATATTATT
   GCTAATAACT AACCAGAGAA GTACGAGCAT TTAACCCTAC CCTATAATAA

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801 TTTCTTG TTC AGGATTTATC TATTGTTGAT AAACAGGCGC GTTCTGCATT
 AAAGAACAAG TCCTAAATAG ATAACAATA TTTGTCCGCG CAAGACGTAA
 851 AGCTGAACAC GTTGT TTTATT GTCGCCGTCT GGACAGAATT ACTTTACCTT
 TCGACTTG TG CAACAAATAA CAGCGGCAGA CCTGTCTTAA TGAAATGGGA
 901 TTGTCGGCAC TTTATATTCT CTTGT TACTG GCTCAAAAAT GCCTCTGCCT
 AACAGCCGTG AAATATAAGA GAACAATGAC CGAGTTTTTA CGGAGACGGA
 951 AAATTACATG TTGGTGTTGT TAAATATGGT GATTCTCAAT TAAGCCCTAC
 TTTAATGTAC AACCACAACA ATTTATACCA CTAAGAGTTA ATTCGGGATG
 1001 TGTTGAGCGT TGGCTTTATA CTGGTAAGAA TTTATATAAC GCATATGACA
 ACAACTCGCA ACCGAAATAT GACCATTCTT AAATATATTG CGTATACTGT
 1051 CTAAACAGGC TTTTTCCAGT AATTATGATT CAGGTGTTTA TTCATATTTA
 GATTTGTCCG AAAAAGGTCA TTAATACTAA GTCCACAAAT AAGTATAAAT
 1101 ACCCCTTATT TATCACACGG TCGGTATTTT AAACCATTAA ATTTAGGTCA
 TGGGGAATAA ATAGTGTGCC AGCCATAAAG TTTGGTAATT TAAATCCAGT
 1151 GAAGATGAAA TTAATAAAA TATATTTGAA AAAGTTTTCT CGCGTTCTTT
 CTTCTACTTT AATTGATTTT ATATAAACTT TTTCAAAAAGA GCGCAAGAAA
 1201 GTCTTGCGAT AGGATTTGCA TCAGCATTTA CATATAGTTA TATAACCCAA
 CAGAACGCTA TCCTAAACGT AGTCGTAAAT GTATATCAAT ATATTGGGTT
 1251 CCTAAGCCGG AGGT TAAAAA GGTAGTCTCT CAGACCTATG ATTTTGATAA
 GGATTGCGCC TCCAATTTTT CCATCAGAGA GTCTGGATAC TAAACTATT
 1301 ATTCACTATT GACTCTTCTC AGCGTCTTAA TCTAAGCTAT CGCTATGTTT
 TAAGTGATAA CTGAGAAGAG TCGCAGAATT AGATTGATA GCGATACAAA
 1351 TCAAGGATTC TAAGGGAAAA TTAATTAATA GCGACGATTT ACAGAAGCAA
 AGTTCCTAAG ATTCCCTTTT AATTAATTAT CGCTGCTAAA TGTCTTCGTT
 1401 GGTTATTCCA TCACATATAT TGATTTATGT ACTGTTTCAA TAAAAAAGG
 CCAATAAGGT AGTGTATATA ACTAAATACA TGACAAAGTT AATTTTTTCC
 1451 TAATTCAAAT GAAATTGTTA AATGTAATTA ATTTTGTTTT CTTGATGTTT
 ATTAAGTTTA CTTTAACAAT TTACATTAAT TAAAACAAAA GAACTACAAA
 1501 GTTTCATCAT CTTCTTTTGC TCAAGTAATT GAAATGAATA ATTCGCCTCT
 CAAAGTAGTA GAAGAAAACG AGTTCATTAA CTTTACTTAT TAAGCGGAGA
 1551 GCGCGATTTT GTGACTTGGT ATTCAAAAGCA AACAGGTGAA TCTGTTATTG
 CGCGCTAAAG CACTGAACCA TAAGTTTCGT TTGTCCACTT AGACAATAAC
 1601 TCTCACCTGA TGTTAAAGGT ACAGTGA CTG TATATTCCTC TGACGTTAAG
 AGAGTGGACT ACAATTTCCA TGTCACTGAC ATATAAGGAG ACTGCAATTC

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1651 CCTGAAAATT TACGCAATTT CTTTATCTCT GTTTTACGTG CTAATAATTT
 GGACTTTTAA ATGCGTTAAA GAAATAGAGA CAAAATGCAC GATTATTAAA
 1701 TGATATGGTT GGCTCTAATC CTTCCATAAT TCAGAAATAT AACCCAAATA
 ACTATACCAA CCGAGATTAG GAAGGTATTA AGTCTTTATA TTGGGTTTAT
 1751 GTCAGGATTA TATTGATGAA TTGCCATCAT CTGATATTCA GGAATATGAT
 CAGTCCTAAT ATAACTACTT AACGGTAGTA GACTATAAGT CCTTATACTA
 1801 GATAATTCCG CTCCTTCTGG TGGTTTCTTT GTTCCGCAAA ATGATAATGT
 CTATTAAGGC GAGGAAGACC ACCAAAGAAA CAAGGCGTTT TACTATTACA
 1851 TACTCAAACA TTTAAAATTA ATAACGTTCG CGCAAAGGAT TTAATAAGGG
 ATGAGTTTGT AAATTTTAAT TATTGCAAGC GCGTTTCCTA AATTATTCCC
 1901 TTGTAGAATT GTTTGTTAAA TCTAATACAT CTAAATCCTC AAATGTATTA
 AACATCTTAA CAAACAATTT AGATTATGTA GATTTAGGAG TTTACATAAT
 1951 TCTGTTGATG GTTCTAACTT ATTAGTAGTT AGCGCCCCTA AAGATATTTT
 AGACAACCTAC CAAGATTGAA TAATCATCAA TCGCGGGGAT TTCTATAAAA
 2001 AGATAACCTT CCGCAATTTT TTTCTACTGT TGATTTGCCA ACTGACCAGA
 TCTATTGGAA GCGGTTAAAG AAAGATGACA ACTAAACGGT TGACTGGTCT
 2051 TATTGATTGA AGGATTAATT TTCGAGGTTC AGCAAGGTGA TGCTTTAGAT
 ATAACCTAAT TCCTAATTAA AAGCTCCAAG TCGTTCCACT ACGAAATCTA
 2101 TTTTCCTTTG CTGCTGGCTC TCAGCGCGGC ACTGTTGCTG GTGGTGTTAA
 AAAAGGAAAC GACGACCGAG AGTCGCGCCG TGACAACGAC CACCACAATT
 2151 TACTGACCGT CTAACCTCTG TTTTATCTTC TGCGGGTGGT TCGTTCGGTA
 ATGACTGGCA GATTGGAGAC AAAATAGAAG ACGCCACCA AGCAAGCCAT
 2201 TTTTAAACGG CGATGTTTTA GGGCTATCAG TTCGCGCATT AAAGACTAAT
 AAAAATTGCC GCTACAAAAT CCCGATAGTC AAGCGCGTAA TTTCTGATTA
 2251 AGCCATTCAA AAATATTGTC TGTGCCTCGT ATTCTTACGC TTTCAGGTCA
 TCGGTAAGTT TTTATAACAG ACACGGAGCA TAAGAATGCG AAAGTCCAGT
 2301 GAAGGGTTCT ATTTCTGTTG GCCAGAAATGT CCCTTTTATT ACTGGTCGTG
 CTTCCCAAGA TAAAGACAAC CGGTCTTACA GGGAAAATAA TGACCAGCAC
 2351 TAACTGGTGA ATCTGCCAAT GTAAATAATC CATTTTACAGC AATTGAGCGT
 ATTGACCACT TAGACGGTTA CATTTATTAG GTAAAGTCTG TTAAGTTCGCA
 2401 CAAAATGTTG GTATTTCTAT GAGTGTTTTT CCCGTTGCAA TGGCTGGCGG
 GTTTTACAAC CATAAAGATA CTCACAAAAA GGGCAACGTT ACCGACCGCC
 2451 TAATATTGTT TTAGATATAA CCAGTAAGGC CGATAGTTTG AGTTCTTCTA
 ATTATAACAA AATCTATATT GGTCATTCCG GCTATCAAAC TCAAGAAGAT

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2501 CTCAGGCAAG TGATGTTATT ACTAATCAAA GAAGTATTGC GACAACGGTT
GAGTCCGTTT ACTACAATAA TGATTAGTTT CTTCATAACG CTGTTGCCAA

2551 AATTTGCGTG ATGGTCAGAC TCTTTTGCTC GGTGGCCTCA CTGATTACAA
TTAAACGCAC TACCAGTCTG AGAAAACGAG CCACCGGAGT GACTAATGTT

2601 AAACACTTCT CAAGATTCTG GTGTGCCGTT CCTGTCTAAA ATCCCTTTAA
TTTGTGAAGA GTTCTAAGAC CACACGGCAA GGACAGATTT TAGGGAAATT

2651 TCGGCCTCCT GTTTAGCTCC CGTTCTGATT CTAACGAGGA AAGCACGTTG
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2701 TACGTGCTCG TCAAAGCAAC CATAGTACGC GCCCTGTAGC GGCGCATTAA
ATGCACGAGC AGTTTCGTTG GTATCATGCG CGGGACATCG CCGCGTAATT

2751 GCGCGGCGGG TGTGGTGGTT ACGCGCAGCG TGACCGCTAC ACTTGCCAGC
CGCGCCGCCC ACACCACCAA TGCGCGTCGC ACTGGCGATG TGAACGGTCG

2801 GCCCTAGCGC CCGCTCCTTT CGCTTTCTTC CTTTCCTTTC TCGCCACGTT
CGGGATCGCG GGCGAGGAAA GCGAAAGAA GGAAGGAAAG AGCGGTGCAA

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2851 CTCCGGCTTT CCCCCTCAAG CTCTAAATCG GGGGATCCCT TTAGGGTTCC  
GAGGCCGAAA GGGGCAGTTC GAGATTTAGC CCCCTAGGGA AATCCCAAGG

2901 GATTTAGTGC TTTACGGCAC CTCGACCTCC AAAAATTGA TTTGGGTGAT  
CTAAATCAGC AAATGCCGTG GAGCTGGAGG TTTTGAAC TAAACCACTA

2951 GGTTACGTA GTGGGCCATC GCCCTAATAG ACGGTTTTTC GCCCTTTGAC  
CCAAGTGCAT CACCCGGTAG CGGGATTATC TGCCAAAAG CGGGAACTG

3001 GTTGGAGTCC ACGTTCTTTA ATAGTGGACT CTTGTTCCAA ACTGGAACAA  
CAACCTCAGG TGCAAGAAAT TATCACCTGA GAACAAGGTT TGACCTTGTT

3051 CACTCAACCC TATCTCGGTC TATTCTTTTG ATTTATAAGG GATTTTGCCG  
GTGAGTTGGG ATAGAGCCAG ATAAGAAAAC TAAATATTCC CTAAAACGGC

3101 ATTTGCGCCT ATTGGTTAAA AAATGAGCTG ATTTAACAAA AATTTAACGC  
TAAAGCCGGA TAACCAATTT TTTACTCGAC TAAATTGTTT TTAAATTGCG

3151 GAATTTTAAC AAAATATTAA CGTTTACAAT TTAAATATTT GCTTATACAA  
CTTAAAATTG TTTTATAATT GCAAATGTGA AATTTATAAA CGAATATGTT

3201 TCTTCCTGTT TTTGGGGCTT TTCTGATTAT CAACCGGGGT ACATATGATT  
AGAAGGACAA AAACCCCGAA AAGACTAATA GTTGGCCCCA TGTATACTAA

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3251 GACATGCTAG TTTTACGATT ACCGTTTCATC GATTCTCTTG TTTGCTCCAG
CTGTACGATC AAAATGCTAA TGGCAAGTAG CTAAGAGAAC AAACGAGGTC

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3301	ACTCTCAGGC	AATGACCTGA	TAGCCTTTTT	AGACCTCTCA	AAAATAGCTA
	TGAGAGTCCG	TTACTGGACT	ATCGGAAAAA	TCTGGAGAGT	TTTTATCGAT
3351	CCCTCTCCGG	CATGAATTTA	TCAGCTAGAA	CGGTTGAATA	TCATATTGAT
	GGGAGAGGCC	GTACTTAAAT	AGTCGATCTT	GCCAACTTAT	AGTATAACTA
3401	GGTGATTTGA	CTGTCTCCGG	CCTTTCTCAC	CCGTTTGAAT	CTTTACCTAC
	CCACTAAACT	GACAGAGGCC	GGAAAGAGTG	GGCAAACCTA	GAAATGGATG
3451	ACATTACTCA	GGCATTGCAT	TTAAAATATA	TGAGGGTTCT	AAAAATTTTT
	TGTAATGAGT	CCGTAACGTA	AATTTTATAT	ACTCCCAAGA	TTTTTAAAAA
3501	ATCCTTGCGT	TGAAATAAAG	GCTTCTCCCG	CAAAAGTATT	ACAGGGTCAT
	TAGGAACGCA	ACTTTATTTT	CGAAGAGGGC	GTTTTTCATA	TGTCCAGTA
3551	AATGTTTTTG	GTACAACCGA	TTTAGCTTTA	TGCTCTGAGG	CTTTATTGCT
	TTACAAAAAC	CATGTTGGCT	AAATCGAAAT	ACGAGACTCC	GAAATAACGA
3601	TAATTTTGCT	AATTCTTTGC	CTTGCCCTGTA	TGATTTATTG	GATGTTAACG
	ATTAAAACGA	TTAAGAAACG	GAACGGACAT	ACTAAATAAC	CTACAATTGC
3651	CTACTACTAT	TAGTAGAATT	GATGCCACCT	TTTCAGCTCG	CGCCCCAAAT
	GATGATGATA	ATCATCTTAA	CTACGGTGGA	AAAGTCGAGC	GCGGGGTTTA
3701	GAAAATATAG	CTAAACAGGT	TATTGACCAT	TTGCGAAATG	TATCTAATGG
	CTTTTATATC	GATTTGTCCA	ATAACTGGTA	AACGCTTTAC	ATAGATTACC
3751	TCAAACATAA	TCTACTCGTT	CGCAGAATTG	GGAATCAACT	GTTACATGGA
	AGTTTGATTT	AGATGAGCAA	GCGTCTTAAC	CCTTAGTTGA	CAATGTACCT
3801	ATGAAACTTC	CAGACACCGT	ACTTTAGTTG	CATATTTAAA	ACATGTTGAG
	TACTTTGAAG	GTCTGTGGCA	TGAAATCAAC	GTATAAATTT	TGTACAACCT
3851	CTACAGCACC	AGATCCAGCA	ATTAAGCTCT	AAGCCATCCG	CAAAAATGAC
	GATGTCGTGG	TCTAGGTCGT	TAATTCGAGA	TTCGGTAGGC	GTTTTTACTG
3901	CTCTTATCAA	AAGGAGCAAT	TAAAGGTACT	CTCTAATCCT	GACCTGTTGG
	GAGAATAGTT	TTCCTCGTTA	ATTTCCATGA	GAGATTAGGA	CTGGACAACC
3951	AGTTTGCTTC	CGGTCTGGTT	CGCTTTGAAG	CTCGAATTAA	AACGCGATAT
	TCAAACGAAG	GCCAGACCAA	GCGAAACTTC	GAGCTTAATT	TTGCGCTATA
4001	TTGAAGTCTT	TCGGGCTTCC	TCTTAATCTT	TTTGATGCAA	TCCGCTTTGC
	AACTTCAGAA	AGCCCGAAGG	AGAATTAGAA	AACTACGTT	AGGCGAAACG
4051	TTCTGACTAT	AATAGTCAGG	GTAAAGACCT	GATTTTTGAT	TTATGGTCAT
	AAGACTGATA	TTATCAGTCC	CATTTCTGGA	CTAAAAACTA	AATACCAGTA
4101	TCTCGTTTTT	TGAACTGTTT	AAAGCATTTG	AGGGGGATTG	AATGAATATT
	AGAGCAAAAG	ACTTGACAAA	TTTCGTAAAC	TCCCCCTAAG	TTACTTATAA

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4151 TATGACGATT CCGCAGTATT GGACGCTATC CAGTCTAAAC ATTTTACTAT
 ATACTGCTAA GCGGTCATAA CCTGCGATAG GTCAGATTTG TAAAATGATA
 4201 TACCCCTCTT GGCAAACTT CTTTGTGCAA AGCCTCTCGC TATTTTGTG
 ATGGGGGAGA CCGTTTTGAA GAAAACGTTT TCGGAGAGCG ATAAAAACAA
 4251 TTTATCGTCG TCTGGTAAAC GAGGGTTATG ATAGTGTTGC TCTTACTATG
 AAATAGCAGC AGACCATTG CTCCCAATAC TATCACAACG AGAATGATAC
 4301 CCTCGTAATT CCTTTTGGCG TTATGTATCT GCATTAGTTG AATGTGGTAT
 GGAGCATTAA GGAAAACCGC AATACATAGA CGTAATCAAC TTACACCATA
 4351 TCCTAAATCT CAACTGATGA ATCTTTCTAC CTGTAATAAT GTTGTTCCGT
 AGGATTTAGA GTTGACTACT TAGAAAGATG GACATTATTA CAACAAGGCA
 4401 TAGTTCGTTT TATTAACGTA GATTTTCTT CCCAACGTCC TGACTGGTAT
 ATCAAGCAA ATAATTGCAT CTA AAAAGAA GGGTTGCAGG ACTGACCATA
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 TTA CTGCGT C AAGAATTTA GCGTATTCCA TTAAGTGTTA CTAATTTCAA
 4501 GAAATTAAAC CATCTCAAGC GCAATTCAT ACCCGTTCTG GTGTTTCTCG
 CTTTAATTTG GTAGAGTTCG CGTTAAGTGA TGGGCAAGAC CACAAAGAGC
 4551 TCAGGGCAAG CCTTATTCAC TGAATGAGCA GCTTTGTTAC GTTGATTTGG
 AGTCCCGTTC GGAATAAGTG ACTTACTCGT CGAAACAATG CAACTAAACC
 4601 GTAATGAATA TCCGGTGCTT GTCAAGATTA CTCTTGATGA AGGTCAGCCA
 CATTACTTAT AGGCCACGAA CAGTTCTAAT GAGAACTACT TCCAGTCGGT
 4651 GCCTATGCGC CTGGTCTGTA CACCGTGCA CTGTCCTCGT TCAAAGTTGG
 CGGATACGCG GACCAGACAT GTGGCACGTA GACAGGAGCA AGTTTCAACC
 4701 TCAGTTCGGT TCTCTTATGA TTGACCGTCT GCGCCTCGTT CCGGCTAAGT
 AGTCAAGCCA AGAGAATACT AACTGGCAGA CGCGGAGCAA GGCCGATTCA
 4751 AACATGGAGC AGGTCGCGGA TTTCGACACA ATTTATCAGG CGATGATACA
 TTGTACCTCG TCCAGCGCCT AAAGCTGTGT TAAATAGTCC GCTACTATGT
 4801 AATCTCCGTT GTACTTTGTT TCGCGCTTGG TATAATCGCT GGGGGTCAAA
 TTAGAGGCAA CATGAAACAA AGCGCGAACC ATATTAGCGA CCCCCAGTTT
 4851 GATGAGTGTT TTAGTGATTT CTTTCGCCTC TTTCGTTTTA GGTTGGTGCC
 CTA CTCA CAA AATCACA TAAAGCGGAG AAAGCAAAAT CCAACCACGG
 4901 TTCGTAGTGG CATTACGTAT TTTACCGTT TAATGGAAAC TTCCTCATGC
 AAGCATCACC GTAATGCATA AAATGGGCAA ATTACCTTTG AAGGAGTACG
 4951 GTAAGTCTTT AGTCCTCAAA GCCTCCGTAG CCGTTGCTAC CCTCGTTCCG
 CATTCAAGAA TCAGGAGTTT CGGAGGCATC GGCAACGATG GGAGCAAGGC

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5001 ATGCTGTCTT TCGCTGCTGA GGGTGACGAT CCCGCAAAAG CGGCCTTTGA
 TACGACAGAA AGCGACGACT CCCACTGCTA GGGCGTTTTTC GCCGGAAACT
 5051 CTCCCTGCAA GCCTCAGCGA CCGAATATAT CGGTTATGCG TGGGCGATGG
 GAGGGACGTT CGGAGTCGCT GGCTTATATA GCCAATACGC ACCCGCTACC
 5101 TTGTTGTCAT TGTCGGCGCA ACTATCGGTA TCAAGCTGTT TAAGAAATTC
 AACAACAGTA ACAGCCGCGT TGATAGCCAT AGTTCGACAA ATTCTTTAAG
 5151 ACCTCGAAAG CAAGCTGATA AAGGAGGTTT CTCGATCGAG ACGTTGGGTG
 TGGAGCTTTC GTTCGACTAT TTCCTCCAAA GAGCTAGCTC TGCAACCCAC
 5201 AGGTTCCAAC TTTCACCATA ATGAAATAAG ATCACTACCG GGCCTATTTT
 TCCAAGGTTG AAAGTGGTAT TACTTTATTC TAGTGATGGC CCGCATAAAA
 5251 TTGAGTTATC GAGATTTTCA GGAGCTAAGG AAGCTAAAT GGAGAAAAA
 AACTCAATAG CTCTAAAAGT CCTCGATTCC TTCGATTTTA CCTCTTTTTT
 5301 ATCACTGGAT ATACCACCGT TGATATATCC CAATGGCATC GTAAAGAACA
 TAGTGACCTA TATGGTGGCA ACTATATAGG GTTACCGTAG CATTTCTTGT
 5351 TTTTGAGGCA TTTCAGTCAG TTGCTCAATG TACCTATAAC CAGACCGTTC
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 TCGACCTATA ATGCCGAAA AATTTCTGGC ATTTCTTTTT ATTCTGTTC
 5451 TTTTATCCGG CCTTTATTCA CATTCTTGCC CGCCTGATGA ATGCTCATCC
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 TCAAACTAA ATTTGCATCG GTTATACCTG TTGAAGAAGC GGGGGCAAAA
 5801 CACTATGGGC AAATATTATA CGCAAGGCGA CAAGGTGCTG ATGCCGCTGG
 GTGATACCCG TTTATAATAT GCGTTCCGCT GTTCCACGAC TACGGCGACC

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5851	CGATTCAGGT	TCATCATGCC	GTTTGTGATG	GCTTCCATGT	CGGCAGAATG
	GCTAAGTCCA	AGTAGTACGG	CAAACACTAC	CGAAGGTACA	GCCGTCTTAC
5901	CTTAATGAAT	TACAACAGTA	CTGCGATGAG	TGGCAGGGCG	GGGCGTAATT
	GAATTACTTA	ATGTTGTCAT	GACGCTACTC	ACCGTCCCGC	CCCGCATTA
5951	TTTTTAAGGC	AGTTATTGGT	GCCCTTAAAC	GCCTGGTGCT	AGCCTGAGGC
	AAAAATTCCG	TCAATAACCA	CGGGAATTTG	CGGACCACGA	TCGGACTCCG
6001	CAGTTTGCTC	AGGCTCTCCC	CGTGGAGGTA	ATAATTGCTC	GACCGATAAA
	GTCAAACGAG	TCCGAGAGGG	GCACCTCCAT	TATTAACGAG	CTGGCTATTT
6051	AGCGGCTTCC	TGACAGGAGG	CCGTTTTGTT	TTGCAGCCCA	CCTCAACGCA
	TCGCCGAAGG	ACTGTCCTCC	GGCAAAACAA	AACGTCGGGT	GGAGTTGCGT
6101	ATTAATGTGA	GTTAGCTCAC	TCATTAGGCA	CCCCAGGCTT	TACACTTTAT
	TAATTACACT	CAATCGAGTG	AGTAATCCGT	GGGGTCCGAA	ATGTGAAATA
6151	GCTTCCGGCT	CGTATGTTGT	GTGGAATTGT	GAGCGGATAA	CAATTTTACA
	CGAAGGCCGA	GCATACAACA	CACCTTAACA	CTCGCCTATT	GTTAAAGTGT
6201	CAGGAAACAG	CTATGACCAT	GATTACGAAT	TTCTAGATAA	CGAGGGCAAA
	GTCCTTTGTC	GATACTGGTA	CTAATGCTTA	AAGATCTATT	GCTCCCGTTT
6251	AAATGAAAAA	GACAGCTATC	GCGATTGCAG	TGGCACTGGC	TGGTTTCGCT
	TTTACTTTTT	CTGTCGATAG	CGCTAACGTC	ACCGTGACCG	ACCAAAGCGA
6301	ACCGTAGCGC	AGGCCGACTA	CAAAGATGTC	GA CTGTATTG	TTTATCATGC
	TGGCATCGCG	TCCGGCTGAT	GTTTCTACAG	CTGACATAAC	AAATAGTACG
				BamHI EcoRI	
				~~~~~	
6351	TCATTATCTT	GTTGCTAAGT	GTGGTGGTGG	AGGATCCGAA	TTCAATGCTG
	AGTAATAGAA	CAACGATTCA	CACCACCACC	TCCTAGGCTT	AAGTTACGAC
6401	GCGGCGGCTC	TGGTGGTGGT	TCTGGTGGCG	GCTCTGAGGG	TGGTGGCTCT
	CGCCGCCGAG	ACCACCACCA	AGACCACCGC	CGAGACTCCC	ACCACCAGAGA
6451	GAGGGTGGCG	GTTCTGAGGG	TGGCGGCTCT	GAGGGAGGCG	GTTCCGGTGG
	CTCCACCGC	CAAGACTCCC	ACCGCCGAGA	CTCCCTCCGC	CAAGGCCACC
6501	TGGCTCTGGT	TCCGGTGATT	TTGATTATGA	AAAGATGGCA	AACGCTAATA
	ACCGAGACCA	AGGCCACTAA	AACTAATACT	TTTCTACCGT	TTGCGATTAT
6551	AGGGGGCTAT	GACCGAAAAT	GCCGATGAAA	ACGCGCTACA	GTCTGACGCT
	TCCCCCGATA	CTGGCTTTTA	CGGCTACTTT	TGCGCGATGT	CAGACTGCGA

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6601 AAAGGCAAAC TTGATTCTGT CGCTACTGAT TACGGTGCTG CTATCGATGG
TTTCCGTTTG AACTAAGACA GCGATGACTA ATGCCACGAC GATAGCTACC

6651 TTTCATTGGT GACGTTTCCG GCCTTGCTAA TGGTAATGGT GCTACTGGTG
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6701 ATTTTGCTGG CTCTAATTCC CAAATGGCTC AAGTCGGTGA CGGTGATAAT
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6751 TCACCTTTAA TGAATAATTT CCGTCAATAT TTACCTTCCC TCCCTCAATC
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6801 GGTTGAATGT CGCCCTTTTG TCTTTGGCGC TGGTAAACCA TATGAATTTT
CCAACCTTACA GCGGGAAAAC AGAAACCGCG ACCATTTGGT ATACTTAAAA

6851 CTATTGATTG TGACAAAATA AACTTATTCC GTGGTGTCTT TGC GTTTTCTT
GATAACTAAC ACTGTTTTAT TTGAATAAGG CACCACAGAA ACGCAAAGAA

6901 TTATATGTTG CCACCTTTAT GTATGTATTT TCTACGTTTG CTAACATACT
AATATACAAC GGTGGAATA CATACATAAA AGATGCAAAC GATTGTATGA

HindIII

6951 GCGTAATAAG GAGTCTTGAT A
CGCATTATTC CTCAGAACTA T

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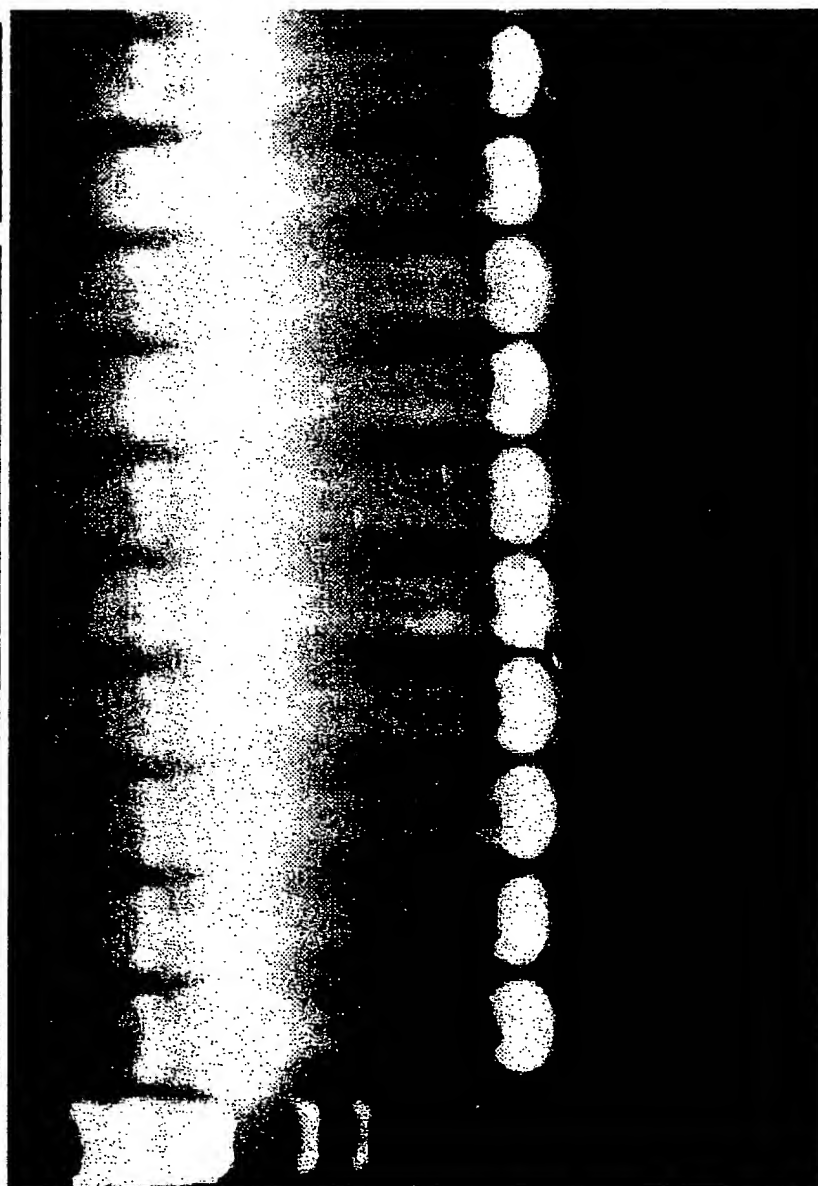
Figure 5



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Figure 6

**co-
M SIP Polyphege transductants transf.**



1 2 3 4 5 6 7 8 9 10

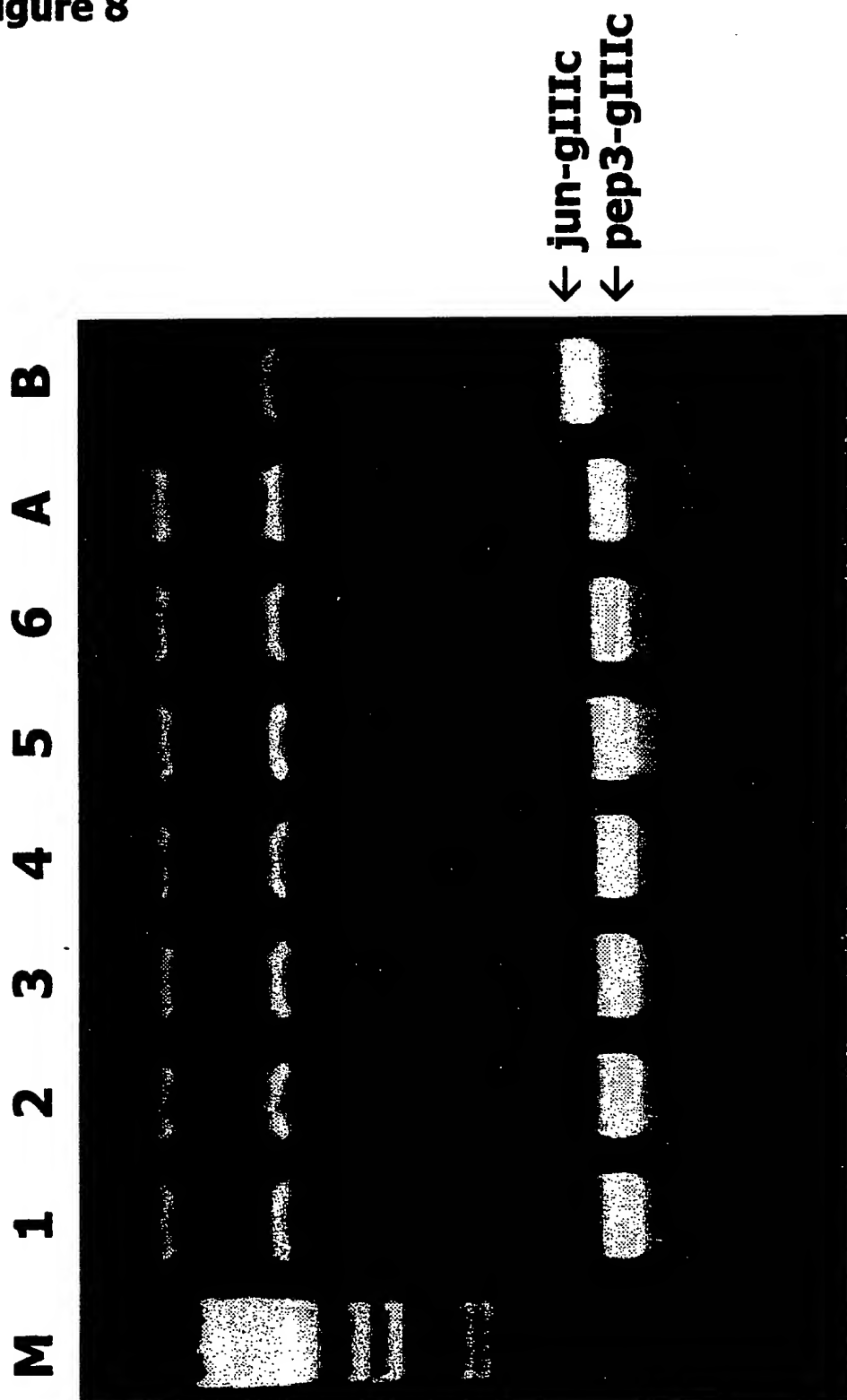
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Figure 7

| pep3/p75ICD | | dilution factor | | transductants
(t.u./ml)* | |
|--------------------|---------------------|------------------------|-------------------|-------------------------------------|--|
| | | | jun/p75ICD | | |
| 1 | pos. control | - | | 6 x 10<sup>5</sup> | |
| - | neg. control | 1 | | 0 | |
| 1 | | 10<sup>2</sup> | | 1.2 x 10<sup>4</sup> | |
| 1 | | 10<sup>3</sup> | | 8.6 x 10<sup>2</sup> | |
| 1 | | 10<sup>4</sup> | | 1.2 x 10<sup>2</sup> | |
| 1 | | 10<sup>5</sup> | | 12<sup>#</sup> | |
| 1 | | 10<sup>6</sup> | | 1.2<sup>#</sup> | |
| 1 | | 10<sup>7</sup> | | 0.12<sup>#</sup> | |

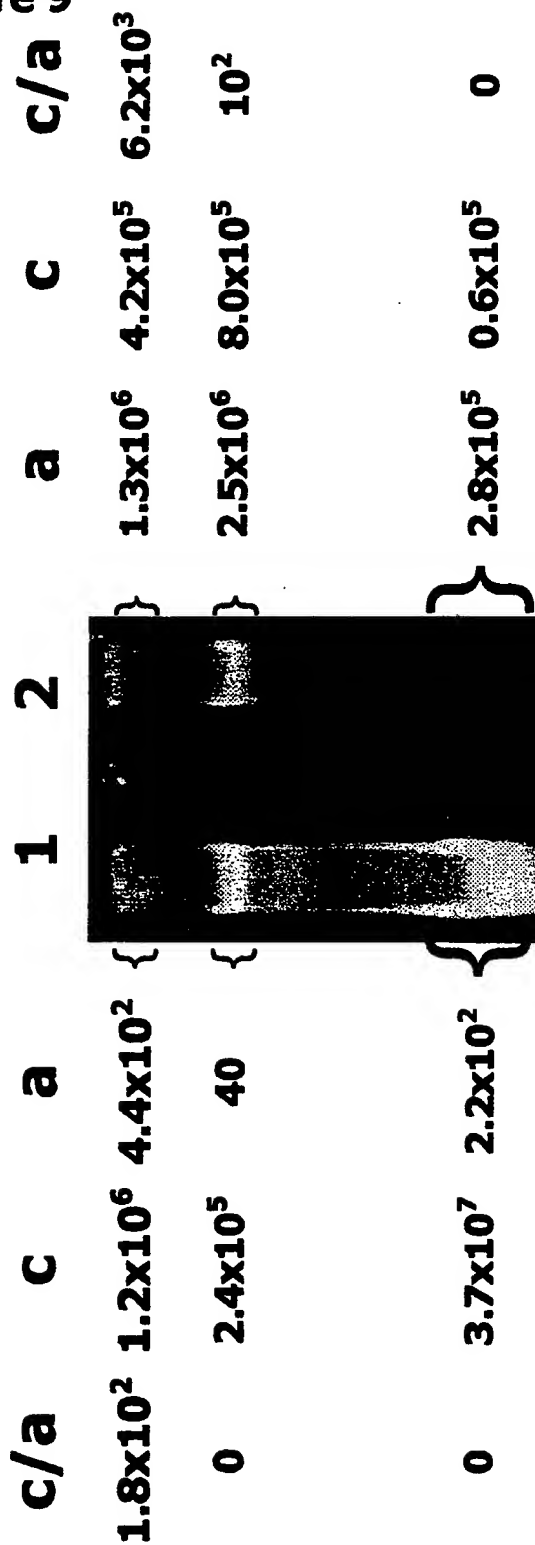
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Figure 8



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Figure 9





INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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ILAG, Vic [PH/DE]; Knorrstrasse 85, D-89897 München
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(74) Agent: VOSSIUS & PARTNER; Siebertstrasse 4, D-81675
München (DE). | | (81) Designated States: CA, JP, US, European patent (AT, BE, CH,
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1 July 1999 (01.07.99) |
| (54) Title: NOVEL METHOD AND PHAGE FOR THE IDENTIFICATION OF NUCLEIC ACID SEQUENCES ENCODING MEMBERS
OF A MULTIMERIC (POLY)PEPTIDE COMPLEX

<p style="text-align: center;">General description of the polyphage principle</p> <p>The diagram illustrates the general description of the polyphage principle. It shows two phage libraries, Library 1 and Library 2, each containing a gene (library1 gene and library2 gene) and an origin of replication (origin of replication1 and origin of replication2). The libraries are shown in a state of infection (a) and a state of replication (b). The process (c) shows the libraries interacting with a target cell, leading to the identification of nucleic acid sequences encoding members of a multimeric (poly)peptide complex.</p>
(57) Abstract
<p>The present invention relates to methods for the identification of nucleic acid sequences encoding members of a multimeric (poly)peptide complex by screening for polyphage particles. Furthermore, the invention relates to products and uses thereof for the identification of nucleic acid sequences in accordance with the present invention.</p> | | |

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/04836

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/10 G01N33/50 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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see page 20, line 3; claims 7-9; examples
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see the whole document
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☒ Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

22 April 1999

Date of mailing of the international search report

07/05/1999

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Fax: (+31-70) 340-3016

Authorized officer

Hoekstra, S

INTERNATIONAL SEARCH REPORT

Internal Application No

PCT/EP 98/04836

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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| A | <p>WO 93 19172 A (CAMBRIDGE ANTIBODY TECH ;MEDICAL RES COUNCIL (GB))</p> <p>30 September 1993</p> <p>see the whole document</p> <p>-----</p> | <p>1-13,
15-43,
45-51</p> |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 98/04836

B x I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 14, 44
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
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Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 14,44

The description, claims 14 and 44 and the drawings are not unambiguous as to which sequence claims 14 and 44 refer to because figure 4 (which these claims refer to) is a mere plasmid map not carrying any sequence data. In consequence at least the claims and drawings are considered not to comply with the prescribed requirements to such an extent that a meaningful search for the subject-matter of claim 14 and 44, using the sequence as characterising part, is not possible

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 98/04836

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date | Patent family
member(s) | Publication
date |
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Information on patent family members

International Application No

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date | Patent family
member(s) | Publication
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